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A description of the symptoms of Acute Oak Decline in Britain and a comparative review on causes of similar disorders on oak in Europe

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Acute Oak Decline (AOD) is a relatively new decline-disease affecting both native oak species (*Quercus robur* and *Q. petraea*) in Britain. The key aim of this study was to describe the symptoms, and signs of AOD, to set a baseline. The second aim was to compare and review the European literature on what appear to be similar disorders on oak. AOD is characterized by four key features: weeping patches more-or-less vertically aligned on oak tree trunks; cracks between bark plates from which dark fluid seeps; inner bark necrosis and the presence (in >90 per cent of cases) of larval galleries of the oak buprestid, *Agrilus biguttatus*, on the phloem–sapwood interface. In this study, it was noted that although larval galleries were present in the inner bark in 19 of 21 trees, the 'D-shaped' exit holes of the adult beetles were seen less frequently on bark plates of affected trees (33 per cent of cases). Similar disorders reported in Europe are compared with AOD in Britain and potential causes of the condition discussed. Based on the unmistakable symptoms, it is hypothesized that AOD is a distinctive, identifiable condition within the broader oak decline syndrome.

Introduction

Dieback and decline in pedunculate (*Quercus robur* L.) and sessile (*Q. petraea* (Matt.) Liebl.) oak has been reported in Britain and continental Europe since the early 1900s (Falck, 1918; Klimesch, 1924; Yossifovitch, 1926; Day, 1927; Osmaston, 1927; Robinson, 1927) although there are some very early reports (mid-eighteenth century, see Thomas, 2008) in Germany. Concern about the increasing deterioration in the health of oak intensified across the whole European region from the 1980s (Delatour, 1983; Oleksyn and Przybyl, 1987; Hartmann *et al.*, 1989; Siwecki, 1989; Hartmann and Blank, 1992; Schütt, 1992; Schlag, 1994; Kowalski, 1996; Gibbs and Greig, 1997; Siwecki and Ufnalski, 1998; Gibbs, 1999) and has continued into the new millennium (Jung *et al.*, 2000; Moraal and Hilszczanski, 2000; Biosca *et al.*, 2003; Van Steenkiste *et al.*, 2004; Thomas, 2008; Denman and Webber, 2009; Marçais *et al.*, 2011). In spite of this concern and the apparent increasing occurrence of oak decline, advances in knowledge on the causes and impact of the condition have been slow to progress and this is partly due to the complex nature of the problem.

Generally, the causes of tree declines are understood to involve assemblages of biotic and abiotic factors (Manion, 1981; Shigo, 1986; Manion and Lachance, 1992), and this is also the view for oak decline (Delatour, 1983; Schlag, 1994; Gibbs and Greig, 1997; Steiner, 1998; Thomas, 2008). However, recently, Denman and Webber (2009) emphasized the need for a closer, more discerning analysis of a new disorder affecting oaks in parts of Britain called 'Acute Oak Decline' (AOD) because trees showed a rapid decline over 3–5 years. This is the first detailed description of the symptoms of this condition in Britain. Similar symptoms on oak in continental Europe have been reported, but detailed descriptions of this are lacking, making it difficult to interpret and compare whether the disorder in Britain is the same as any of those reported in Europe. Furthermore, there is a lack of consensus amongst the European scientists on the causes of these similar condition(s) (Krahl-Urban *et al.*, 1944; Jacquiot, 1949, 1950, 1976; Donaubauer, 1987; Cech and Tomiczek, 1986; Hartmann *et al.*, 1989; Hartmann and Blank, 1992; Kehr and Wulf, 1993; Kowalski, 1996; Gibbs and Greig, 1997; Gibbs, 1999; Biosca *et al.*, 2003; Van Steenkiste *et al.*, 2004). In light of this confusion, an explicit description of the symptoms of AOD in Britain is required and will establish a baseline that can be used for future comparison. Thus, the overall objectives of this study are as follows: (1) to present a detailed description of the visible external and internal symptoms and signs that characterize trees symptomatic of AOD in Britain and (2) to critically review possible causes of similar conditions reported in continental Europe.

Methods

An observational study on the symptoms and signs that characterize AOD in Britain was carried out.

Site and tree selection

Sites were selected randomly (by drawing names from a hat) from reports made to the Disease and Diagnostic Advisory Services (DDAS) at Forest Research (FR), Alice Holt, in Surrey. Twenty-one trees spread over 16 sites were used in this study. On each site, only one or two trees were used for

study because sampling was destructive involving the removal of a fully barked panel from symptomatic trees and this required permission from the owners of the trees, but also working the samples in the laboratory was very time-consuming, so only a manageable number of samples could be processed at any one time. Sites were usually visited twice or three times before samples were taken to enable appropriate selection of trees to cover a range of symptom severity (Table 1).

Sampling and characterization

Notes on site history, characteristics and management practices were made. The location of each tree was recorded using GPS (Garmin GPSmap 60CSx – Global Positioning Systems). Photographs documenting the characteristics of the external symptoms were taken. Signs, i.e. evidence of pests such as D-shaped exit holes indicative of the bark-boring insect *Agrilus biguttatus* Fabricius or pathogens – for example fungal fruiting structures, were recorded. Crown condition was assessed on a scale of 1–5 with 1 being very poor and 5 being very healthy. The Hessian method of crown morphology assessment of old oaks (Hessian Forest Research Institute Hahn, Münden, Germany) was used as a guide for winter assessment when the crown was bare, and the Forestry Commission's Field Book 12 (Innes, 1990) was used as a guide in summer when trees were in full leaf. Diameter at breast height (DBH, i.e. 1.3 m) was measured using a Forestry Suppliers metric diameter tape. Tree height was estimated, and approximate tree age was determined through girth conversion according to the method described by White (1998). Severity of the condition was assessed by categorizing the number of bleeding points on stems: >20 stem bleeds scored advanced/severe; between 10–20 bleeds was moderate and <10 bleeds was considered a light infection. The aspect (i.e. compass direction) of the weeping patches was noted.

Once notes about external features were made, either a chain saw or a sharp, surface-disinfected chisel was used to remove a fully barked panel taken to a depth in the heartwood, and ranging in size from ~20 × 15 cm to a maximum of 60 × 45 cm (L × B) and a depth of 5–7 cm. The panel was selected to include one or more bleed points, and both bark and wood tissues. The panel was placed in a clean polythene bag, kept cool and taken to the laboratory for processing. All sampling equipment was thoroughly surface-disinfected between samples.

In the laboratory, each sample was photographed prior to processing. The panel was lightly surface-disinfected by spraying 70 per cent ethanol on both surfaces and allowing it to air dry. A carpenter's draw knife was then used to systematically pare through the stem tissues in the following way: the panel was placed on the bench top. The blade of the draw knife was placed at an acute angle to the surface of the panel pushed into the bark and repeatedly drawn towards the operator, shaving a layer of bark away with each action. In this way, the nature and extent of the lesions were obtained in a 3-dimensional view. The lesions in each of the tissue types were described and photographed in each layer of tissue. Small samples could not be investigated using a draw knife because of their size so they were clamped in a vice and split along the inner–outer bark and inner bark–vascular cambium interfaces, using a chisel. Then, using a scalpel, the tissue was shaved back to reveal the extent of the lesions. Symptoms observed were documented and photographed, and signs of insect activity were recorded. Phloroglucinol-stain testing was applied to the callus-like repair tissue to test for lignification. Although isolations for detection of microbes associated with the necrotic tissue were made, these results are reported elsewhere (Denman *et al.*, 2013, 2014, unpublished).

Results

Site and tree selection

All the study sites were located in England because no reports were received from Scotland or Wales. All sites were on clay-rich, slowly permeable, seasonally water-logged soils with the exception of site S, which was on a deeply permeable fine loamy soil (Table 1). This site was once a sand pit, the sand was dug out and used for construction. The height of the symptomatic trees ranged between 16 and 26 m. The smallest DBH recorded was 34 cm, the largest was 81 cm and the trees tended to be at maturity ranging between 50 and 200 years in age. Symptoms were present on both *Q. robur* and *Q. petraea* (Table 1).

Characterization: external symptoms

The following symptom description is based on a collation of observations on the 21 trees studied. Symptoms with their descriptions are categorized beginning with stem bleeds, followed by bark cracks, crown condition, callus repair and *Agrilus* activity. This pattern of symptom categorization is also used in the internal symptom descriptions that follow. It should be noted that in this study, the description is based on observation at sampling.

Stem bleeds

The weeping patches occurred exclusively on the tree trunk. The lowermost bleed points occurred at 30–50 cm above ground and could extend up the main stem beyond the scaffold branches (15–20 m above the ground); however, bleed points were most often found 1–5 m above ground. Severely affected trees were most diagnostic and easily identified (Figures 1 and 2; Table 1). The distance between visible bleeds in the vertical direction usually ranged between 5 and 22 cm, and that in the horizontal plane bleeds could be 5–14 cm apart. As the disease worsened, new exudation points appeared either above or below the original bleed point.

Active stem bleeding was usually seen early in spring (March–April) through to June and again in the autumn (October–November), but there was variation around this. None-the-less active bleeding was not usually present during the peak summer months (July–September) or mid-winter months (December–February). When bleeding occurred, the fluid was typically clear (translucent), but dark in colour (Figure 3). However, if yeast colonization of the fluid occurred, it became cloudy.

No particular aspect to the position of the bleeds was evident. Trees with abundant stem bleeds (i.e. severe symptoms) were frequently affected on multiple aspects.

Crown condition

This varied considerably amongst symptomatic trees with no clear evidence of a significant pattern associated with severity of stem symptoms. Applying a Gamma statistic to the 21 trees of the data set failed to identify a significant positive correlation between crown condition and severity class ($P = 0.24$). A larger sample size and monitoring to record canopy condition would help clarify the relationship between crown condition and severity of stem symptoms.

Bark cracks

In the earliest stage of symptom development, the bark was still intact but a little fluid was present. When cracks were present, their length ranged from 3 to 22 cm. However, cracks longer than

Table 1 Locality, characteristics, soil type, symptoms and condition of trees studied

Site county[a]	Tree No.	DBH[b] (cm)	Estimated height (m) Age (y)[c]	Soil type[d]	Severity of external stem symptoms, status of development and presence of *Agrilus* exit holes	Aspect of bleeds	Galleries present in inner bark	Crown condition and ranking (1–5)
AT (Sal)	1	71	24 100	711m SALOP-	Severe[e], advanced[f], developing. Multiple bleeds (≫20). 0.5 m->15 m. Very few *Agrilus* exit holes evident	All	Yes	Very poor, tree almost dead (1)
PW (Brk)	2	81	26 125	711h WICKHAM 4	Severe, developing[g]. Multiple bleeds (>20). 1.5–15 m. *Agrilus* exit holes evident	All, S-more severe	Yes	Moderate, tree still alive, assessed in winter (3)
BH (Es)	1	53	23 125	714c OAK2	Light, healing. Few bleeds (<10). Bleeds present 1–6 m. No exit holes evident. Bleeds not active but occluding	E	Yes	Poor (2)
	4	55	21 130		Moderate, developing, 10–20 bleeds. No exit holes seen	All	Yes	Good (4)
BW (Es)	1	56	20 130	714c OAK2	Severe, healing. No active bleeds. Old bleeds occluding with lignified-callus-like tissue. No *Agrilus* exit holes	All	Yes	Poor (2)
	2	72	25 190		Severe, advanced. Multiple bleeds (>20). *Agrilus* exit holes present	E; SE	Yes	Moderate (assessed in winter) (3)
CE (Ox)	1	67	18 100	711f WICKHAM 2	Severe and advanced but healing. Multiple bleeds up to 8 m on stem. Bleeds active in 2009 but inactive in 2010. Lots of *Agrilus* exit holes. Large old wound on west. Some of the bleeds are occluding	N; S; W	Yes	Good (4)
FOD (Gl)	9	87	19 200	711p DUNKESWICK	Light, developing attack. A single cluster of 5–10 bleed points at 2–3 m. Exit holes not noted	W	Yes	Poor (2)
GOR (Le)	1	55	22 80	431 WORCESTER	Light, healing. One of the first samples. No note of *Agrilus*	SE	Yes	Good (4)
H (Sr)	1	75[h]	25 120	711h WICKHAM 4	Severe, developing. No exit holes, lots of galleries. Multiple bleeds (>20). 1.5–15 m	N; E; S	Yes	Poor (2)
HO (Hrt)	3	60[h]	22 145	712c WINDSOR	Moderate, advanced, developing. Exit holes not noted	ND	Yes	Moderate (winter) (3)
	4	68[h]	23 175		Moderate, advanced, developing. Exit holes not noted	ND	No	Moderate (winter) (3)
M (Wo)	1	85	25 150	711b BROCKHURST 1	Severe, developing. Multiple bleeds (>20). 1.5–15 m. Lots of exit holes	All	Yes	Poor (2)
OUT (Le)	1	46	26 80	711I CLAVERLEY	Moderate, developing. Exit holes not noted	All	Yes	Moderate (2)
	3	35	21 60		Moderate, developing. Exit holes not noted	All	Yes	Very poor (1)
PIR (Wo)	16	75	ND 120	572F WHIMPLE 3	Light, healing, old incursion on epicormic tree. Only 2 bleeds, wound on east side. No *Agrilus* Exit holes	N; S	Yes	ND
S (Sr)	1	65	ND 110	573a WATERSTOCK	Severe, advanced. Multiple bleeds >>20. *Agrilus* exit holes evident	All	Yes	Poor (2)
SPW (Le)	1	70[h]	25 110	712b DENCHWORTH	Severe, advanced, progressive. Multiple bleeds >>20. No note about *Agrilus* exit holes	All	Yes	Poor (2)

Continued

Table 1 *Continued*

Site county[a]	Tree No.	DBH[b] (cm)	Estimated height (m) Age (y)[c]	Soil type[d]	Severity of external stem symptoms, status of development and presence of *Agrilus* exit holes	Aspect of bleeds	Galleries present in inner bark	Crown condition and ranking (1–5)
SB (Ox)	1	55	16 80	711F WICKHAM 2	Light, healing, not typical, < 10 bleeds 1.2–3 m up stem. No exit holes visible	N; S	Yes	Moderate (3)
	3	67	22 100		Severe, advanced but evidence of healing. Multiple bleeds ≫20. Bleeds not active. Very few exit holes present (<5)	NW; SE	Yes	Moderate (3)
WW (Sf)	55	57	ND 135	582d HORNBEAM 3	Light < 10 bleeds. No exit holes. Panel low, lesions occluding with lignified-callus-like tissue. Has progressed quickly (severe in 2011 lots exit holes)	N; E	No	Moderate (3)

[a]Brk, Berkshire; Es, Essex; Gl, Gloucestershire; Hrt, Hertfordshire; Le, Leicestershire; Ox, Oxfordshire; Sal, Shropshire; Sf, Suffolk; Sr, Surrey; Wo, Worcestershire.
[b]Diameter at breast height, i.e. 1.3 m.
[c]Age estimated according to White (1998) – rounded off to the nearest 5 years.
[d]Avery (1980)
[e]Severe symptoms relates to the number of stem bleeds per tree being >20.
[f]Advanced symptoms means that symptoms have been present for >2 seasons, healing not visible.
[g]Developing symptoms means that symptoms are still developing and have only been present for 2 seasons.
[h]Data not recorded at the time but estimated from scaled photographs.
ND, no data.

15 cm were rare. Cracks on the outer bark were characterized by blackened ragged margins. Bark cracks probably only develop when the underlying tissues decay. Once the live bark underlying the outer bark had decayed a hollow area formed underneath the crack and the gap between the bark plates widened (Figure 4).

Agrilus

Signs of colonization by *A. biguttatus* larvae were concealed beneath the bark and were thus not outwardly visible on trees *in situ*. However, once larvae pupated and adult beetles emerged successfully, 'D-shaped' exit holes were left on the bark plates of tree stems indicating previous colonization (Figure 5). These exit holes were 3–4 mm long along the flat edge of the 'D' and 2–2.5 mm in height at the widest part. Of the 21 trees recorded in this study, only one-third (7 trees) had clearly visible *Agrilus* exit holes (Table 1). When exit holes were present, only a small number per tree was seen (frequently <10 and seldom >50), with the exception of one case where the tree was in the advanced stages of a severe attack and nearing death, and exit holes were more abundant.

Callus repair

The first visible symptoms of repair to damaged bark were evident as lignified-callus-like tissue, which stained red when phloroglucinol was applied in the laboratory. In the field, close examination of tissue behind the outer bark around the crack revealed whether necrosis was still developing or whether healing was under way. Of the 21 symptomatic trees inspected in this study, one-third (7 trees) showed a host healing response. In the repair process, the lignified-callus-like tissue built up along the outer edges of the lesion and took on a darkened dull-marbled appearance as it rolled over the surface of the dry wood and occluded the lesion. When occlusion occurred, the outer bark was pushed outwards with the exposed rolls of lignified-callus-like tissue puckered around a central suture-like line (Figure 6). Subsequently, the pieces of outer bark loosened by the expanded lignified-callus-like tissue fell away leaving a pock-marked appearance to the stem (Figure 7). In some cases, although the original bleed point had been occluded by lignified-callus-like tissue, the bleeding had reactivated from the original lesion breaking through the newly formed phloem. In other cases, re-infection of previously attacked and healed trees was identified (Figure 8).

Characterization: internal symptoms

Symptoms within the outer bark (rhytidome/phellem/cork)

Stem bleeds

Figure 9a illustrates stem bleeds on the outer surface of the outer bark/rhytidome, but once the outermost cells of the outer bark were removed from the bark plates using the draw knife, it was clearly evident that the dark fluid seeped along the crevice lines between the bark plates both in vertical and horizontal directions, but mostly vertically (Figure 9b). Thus, a black spreading damp stain developed in the cork cells (Figure 9b). Although there was a good match with the external bleed point and visible tissue necroses directly underneath (compare Figure 9a–c), there were

Figure 1 External symptoms of AOD showing position of weeping patches on tree trunks.

Figure 2 Close-up of external symptoms of AOD featuring dark weeping patches on tree trunks.

Figure 3 Clear dark fluid seeping from cracks between bark plates on the stem of a mature oak with AOD.

Figure 4 Gap between bark plates widening to form a crack because the underlying tissues are necrotic and decaying.

Figure 5 D-shaped exit hole of an *Agrilus biguttatus* adult beetle. Scale bar: ~4 mm.

Figure 6 A fully occluded AOD lesion – closed over by lignified-callus-like tissue.

often additional necrotic patches in the inner bark that were not yet visible externally.

Agrilus

In a very few instances, larvae of *A. biguttatus* were found in pre-pupal chambers in the cork layers of the bark plates, but this was relatively rare. If seen, larvae were lying in a folded position ready for pupation. Empty pupal chambers were encountered infrequently in the rhytidome, and they were always linked to D-shaped exit holes.

Symptoms in the phloem (inner bark)

Lesions

Under the rhytidome (outer bark), the colour of healthy live inner bark was pale cream with the ray cells presenting themselves as slightly darker vertical streaks. Exposed phloem oxidized to a

Figure 7 A mature oak with occluded AOD lesions. The outer bark has fallen away from the occluded areas giving a pock-marked appearance to the stem.

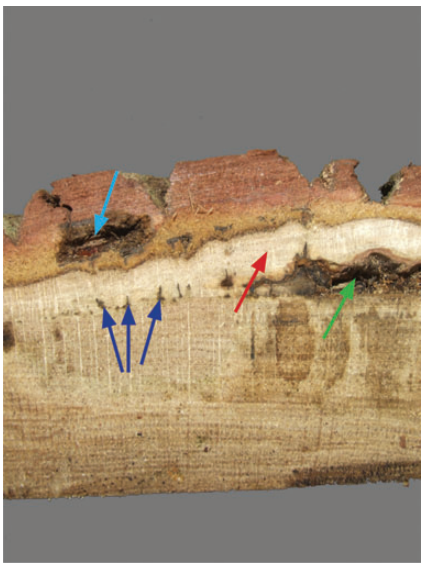

Figure 8 X-S through AOD lesions on tree trunk: occluded area (red arrow), lesion and cavity formed in a subsequent attack (sky blue arrow), original AOD lesion (green arrow) and *Agrilus* larval galleries (dark blue arrow).

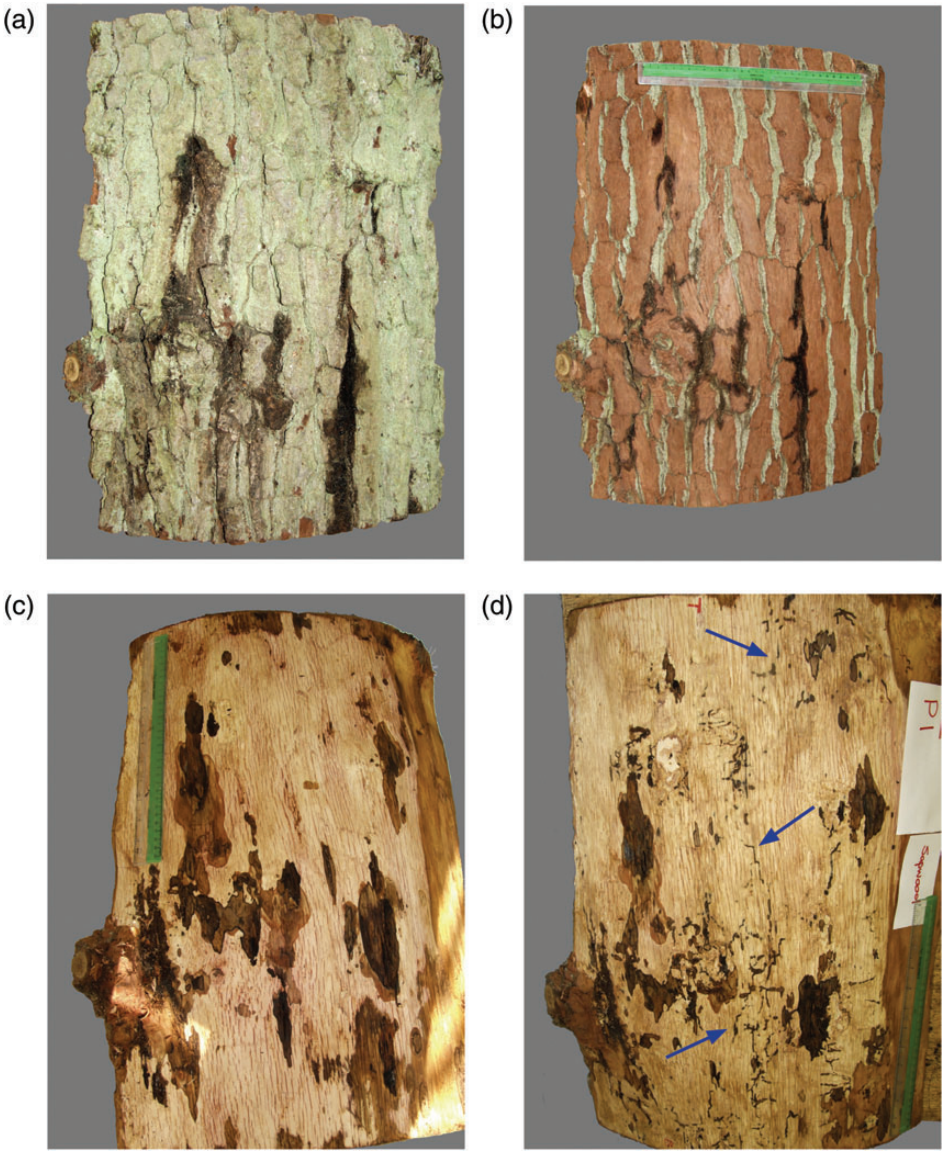

Figure 9 (a) External symptoms of AOD on bark panel removed for further analyses. (b) Stem bleeds in the sub-surface outer bark showing good correlation with external bleed points (a). (c) Lesions in the inner bark. Note the greater extent of necrosis than the weeping patch (a and b) indicates. (d) Lesions still present but reducing the outer sapwood and cambium layers where the *Agrilus* galleries become evident (dark blue arrows).

darker colour quite rapidly. In symptomatic trees, lesions were present as patches of dark, irregularly oval-shaped, frequently wet and slimy necrotic tissue in the live inner bark. Lesions originated around the rays and spread outwards from these points. Older lesions showed colour zonation, being bounded by a black border that demarcated the extent of first necrotic activity (Figure 10). Lighter brown 'breakout' zones extending beyond the black margins of the lesions were often present suggesting sequential lesion extensions with the most recent being the palest in colour appearing as light pinkish-water-soaked areas on the outermost margins of the lesion (Figure 10). When the tissue was totally degraded, a cavity was formed (Figure 10). Individual lesion size

varied; the largest were >20 cm long and 8 cm wide. Expanding lesions sometimes coalesced to form a more-or-less continuous irregularly shaped area of necrosis. Lesions developed through the inner bark towards the sapwood (Figure 9c and d).

Cavities

When inner bark decay had advanced to the sapwood, a cavity was created, which can be seen clearly in cross section of a tree stem (Figure 11). When symptoms were severe, the damage to the inner bark and sapwood was extensive (Figure 9c and d). Also visible in cross sections were small black spots or short dash marks indicating *Agrilus* larval feeding along the phloem–sapwood interface (Figure 8). Short darkened breach-lines sometimes occurred along some of the rays indicating lesion development along a live tissue (parenchyma) pathway.

Agrilus

Galleries of *A. biguttatus* were absent from the outer layers of the phloem. However, when the phloem–sapwood boundary was exposed, signs of *Agrilus* larval activity were visible (Figure 9d). Galleries were evident in 19 out of the 21 trees studied present as a network of fine-to-coarse dark lines with clearly defined margins (Figures 12 and 13). The galleries tended to avoid the lesions but if larvae came close to or made contact with a lesion, the gallery margins lost their sharp well-defined edges and became diffuse (Figure 14). Generally, three gallery width-classes could be approximated: 1 mm or less; ~2 mm wide; ~3–5 mm wide, probably corresponding to different instar phases of the larvae. The smallest galleries were present in a zigzag pattern, turning in acute angles as they crossed the cambium; medium-sized galleries were often, but not always, orientated in a vertical direction and frequently demonstrated a short horizontal step-like pattern, giving them a distinctive staircase appearance. The largest galleries tended to follow a sinuous pattern, twisting back and forth often in a relatively small concentrated area (Figure 12). The largest galleries were the most frequently seen; the smallest ones had usually disappeared or been covered with lignified-callus-like tissue (Figure 15). Sometimes, a radial gallery pattern was seen and appeared to be caused by two or more larvae coming close together and then turning away from each other. The galleries often appeared to have been formed one, two or more seasons previously and were frequently becoming covered with lignified-callus-like tissue (Figure 15). Live larvae in their galleries were usually not encountered although they were seen on one occasion (Figure 12). The incidence and intensity of gallery formation was generally much lower by comparison with that evidenced in trees dying primarily of other causes (for example, *Armillaria* Fries attack) and not exhibiting any symptoms of stem bleeding (Figure 16). In the *Armillaria* examples, all the size classes of galleries could be seen clearly and there was no lignified-callus-like tissue present.

Callus

If lignified-callus-like tissue was present, it was encountered at the sapwood–cambial interface and appeared hard and white. In cross section, the lignified-callus-like tissue appeared to 'heap up' or roll-over the lesion or cavity from the margins (Figure 17), creating a bulge on the stem when it occluded (Figure 18). In circumferential view, exposed phloem tissue had a wavy appearance in the vertical plane (Figure 18). The lignified-callus-like tissue was extraordinarily hard and was almost impossible to pare away with a draw knife.

Symptoms in the sapwood (cambial zone)

Lesions

Lesions extended down to the sapwood and sometimes continued through several sapwood rings but did not enter the heartwood. The necrotic area on the sapwood was not as extensive as it was in the phloem (Figure 9d).

Agrilus

Damage caused by *Agrilus* larvae appeared to be restricted to the cambial zone. Galleries usually did not extend into the heartwood.

Discussion

In Britain, AOD is a relatively newly discovered condition of mature oak, and Denman and Webber (2009) suggested that careful discerning inspection and knowledge of disease symptomatology on oak would aid identification of this condition in the field and reduce the chance of misidentification of trees affected by other agents but demonstrating similar symptoms. A clear description of the symptoms of AOD was required to avoid this problem because some of the symptoms of AOD, for example stem bleeding, may also be caused by other agents. In this study, there was a high level of consistency in the symptoms evident both externally and internally, and this gives a degree of confidence that the symptoms of AOD are distinctive. The 21 trees used in this study were established (>50 years old) native oak species (*Q. robur* and *Q. petraea*), and the description of AOD given here is based on this scenario, but the possibility of AOD occurring on younger trees or on other tree species should not be discounted.

There are four key descriptors that identify AOD: (1) weeping patches on oak stems, (2) cracks in the outer bark from which dark fluid seeps, (3) irregularly oval-shaped lesions in the inner bark and/or cavities behind the outer bark around the seepage point and (4) the usual presence of galleries of the buprestid *A. biguttatus* in the newest phloem and sapwood. Below, each diagnostic feature is further discussed and compared with similar symptoms caused by other agents.

Diagnostic symptom (1): weeping patches on oak stems/stem bleeds

A key external symptom of AOD is the weeping patches, often termed stem bleeds, on the trunks of oak trees. The particular, mostly vertical, distribution and arrangement of the bleeds on the stems of established oak trees is diagnostic. This vertical arrangement of weeping patches may have something to do with spread of pathogenic agents in the sapwood (xylem transported), considering that the necrosis penetrated the active sapwood layers. Once necrogenic agents are confirmed, it will be interesting to explore and test this idea experimentally.

All trees investigated in this study showed the known symptoms of AOD, and none of the symptomatic trees were confused with

Figure 10 Inner bark lesion: light areas = fresh break-out zones of necrosis (sky blue arrows), dark zone lines mark the seasonal halt of lesion expansion (green arrow), total tissue degradation forming a cavity (red arrow).

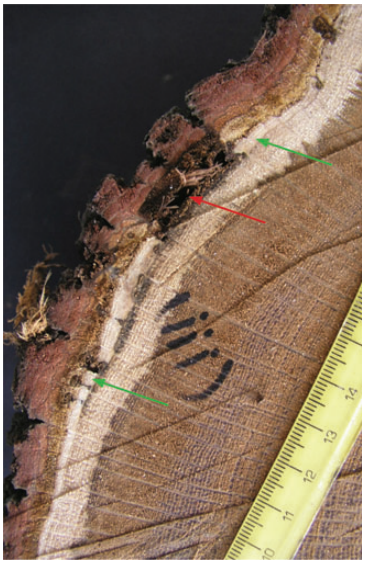

Figure 11 X-S of an AOD lesion demonstrating tissue degradation leading to cavity formation (red arrow) between the outer bark and sapwood layers and lignified-callus-like tissue (green arrows).

Figure 12 Inner bark showing (dark blue arrows) *Agrilus biguttatus* galleries and necrotic tissue. (Red arrow) drowned larva; (green arrow) live larva in unaffected cambial–sapwood tissue.

Figure 13 *Agrilus* larval galleries with clearly defined margins (dark blue arrow); and necrotic tissue (red arrow).

Figure 14 *Agrilus* larval galleries with diffuse margins (dark blue arrow).

Figure 15 Galleries of *Agrilus* larvae healed over by lignified-callus-like tissue (dark blue arrows).

trees that had stem bleeds caused by other agents. However, stem bleeding is a generic host response to tissue damage in a number of tree species and can be brought about by different damaging agents (Gibbs and Greig, 1997), for example certain fungal or fungal-like pathogens such as *Armillaria* and *Phytophthora* are known to cause stem bleeding on oak (Day, 1927, 1938; Moreau and Moreau, 1952; Mircetich *et al.*, 1977; Robin, 1992; Strouts and Winter, 1994). To help avoid misdiagnosis, we compare symptomology of disease conditions caused by other pathogens that might be confused with AOD below.

Figure 16 High incidence and concentration of *Agrilus* larval galleries (dark blue arrows) on oak attacked by *Armillaria*.

Figure 17 X-S of an occluded lesion. The callus-like tissue has formed over the lesion resulting in a T-shaped scar that led to the generic name T-Disease.

Figure 18 Circumferential view of an occluded lesion showing convex tissue profile and wavy appearance of callus-like tissue.

Figure 19 Oval-shaped exit hole of an adult long-horn beetle (*Cerambycidae*). Scale bar: ~5 mm.

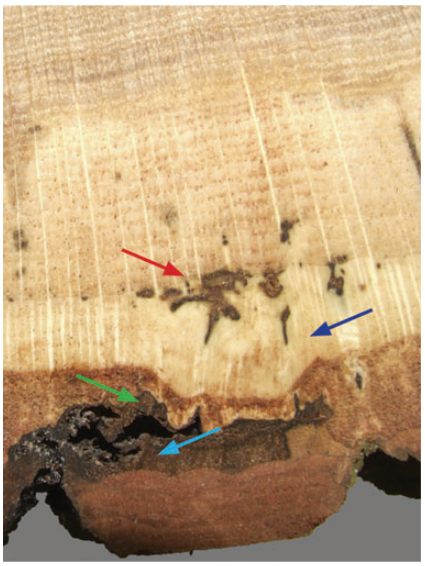

Figure 20 X-S of stem of advanced AOD-symptom oak. Galleries on previous cambium/sapwood layer (red arrow), callus-like tissue overlaying (dark blue arrow), lesion in new phloem (green arrow) fluid staining in outer bark (sky blue arrow).

Armillaria *stem bleeding*

Stem bleeds caused by pathogenic *Armillaria* species on oak differ from those caused by AOD because they first appear around the base of the stem, clustering with the bleed points aligning more or less horizontally or tapering to an 'A-frame' shape. AOD stem bleeds usually develop higher up the stem (1–5 m), and they align more or less vertically. As the *Armillaria* hyphae extend up the stem, the bleed points migrate upwards with the underlying mycelial front leaving no trace of bleeding in the previous lower positions, whereas the bleed points in AOD tend to be persistent and have a stable position over time with cracks enlarging and new points developing close to the original bleeds as the inner bark necrosis expands. Cracks do not develop between the bark

plates in *Armillaria* infection, and the fluid exuded by *Q. robur* and *Q. petraea* tends to be a clear honey-tobacco brown colour at first, darkening later, whereas AOD fluid is dark, translucent blackish-brown drying to form shiny black glassy beads.

If the outer bark is lifted in destructive sampling and the advancing front of the attack is uncovered, white mycelial fans that confirm the presence of *Armillaria* are present, and *Agrilus* galleries may be evident if the beetles have colonized the failing tree. If this is the case, there is often a very high density of galleries in close proximity to the *Armillaria*-attacked tissue. *Armillaria*-colonized inner bark is usually pale in colour (grey or cream) and dry. But with AOD, underneath the outer bark, the infected tissue is dark, moist, friable and necrotic, or a cavity may be present in the inner bark if the tissue has been sufficiently decayed. *Agrilus* galleries are usually present in the cambial zone and are often at a low density.

It is important to remember that some species of *Armillaria* are considered harmless secondary colonizers of weakened oak tissue (Strouts and Winter, 1994) that do not induce stem bleeding, but other species are primary pathogens. In Britain, more information about the identity and pathogenicity of the different *Armillaria* species on oak is urgently required. We conclude that the stem bleed symptoms induced by pathogenic *Armillaria* species on pedunculate and sessile oak are sufficiently distinguishable from AOD symptoms for diagnostic purposes.

Phytophthora *stem bleeding*

Stem bleeds caused by *Phytophthora* species (e.g. *P. cinnamomi* Rands and *P. cambivora* (Petri) Buisman) on oak might be confused with the early stages of AOD attack. Visible *Phytophthora* lesions on native oak in Britain are usually first seen around the collar region of trees, i.e. at the soil surface interface, and migrate up the stem leaving no trace of the initial bleed points but may be seen higher up the stem (e.g. at 1 m). The colour of the fluid seeping from the stem is usually dark, purplish black (hence the common name 'ink disease'), but it does not emanate from highly noticeable developing cracks in the outer bark although small cracks may occur in some cases (Strouts and Winter, 1994).

If the lesion has progressed some distance up the stem, it could be mistaken for an early stage of AOD and a non-destructive 'lateral-flow-device' test (LFD) for *Phytophthora* may prevent destructive sampling. In the absence of a LFD-positive test, in Britain a non-invasive swab of the fluid may be sent to the FR laboratory to test for the presence of bacteria associated with AOD. However, if no fluid is present and there is concern that the lesion might indicate the early stages of AOD, destructive sampling and laboratory analyses of the lesions in the inner bark are needed for further diagnosis.

Phytophthora attack usually causes quite rapid tissue necrosis and unlike AOD does not physically degrade the phloem to sawdust or leave cavities behind the outer bark. Rather the mycelium moves quickly through the inner bark killing it functionally but leaving dead tissue intact behind the outer bark. Therefore, the presence of cavities would indicate AOD as opposed to *Phytophthora* attack.

The shape of *Phytophthora*-induced lesions in the phloem is different from AOD lesions – they are usually larger and more extensive and do not have the irregular oval or oblong shapes centred on the rays, typical of AOD lesions. Furthermore, the inner bark beneath *Phytophthora*-induced weeping patches is usually water-soaked, often orange-brown in colour and may be mottled or demarcated by zone lines and the wood is stained blue-black (Strouts and Winter, 1994). Fresh AOD lesions tend to have a wet, shiny quality to them and *Agrilus* galleries are usually present.

If a tree can overcome and resist a *Phytophthora* attack, which it frequently does (Robin, 1992; Marçais *et al.*, 1996), the necroses and bleeds do not spread far up the stem and a 'flame- or A-shaped' healing scar may form (Strouts and Winter, 1994). In contrast, AOD lesions occur up the stem into the tree crown and are usually initiated 1–5 m up the stem; the bleeds are fairly regularly spaced along the stem with a vertical aspect to their appearance. Furthermore, *Agrilus* galleries do not appear to be associated with *Phytophthora* attack on oak or have not previously been noted as such but occur frequently in association with AOD. Thus, both internal and external symptoms are important when distinguishing between AOD and *Phytophthora*-induced stem bleeding.

Agrilus *stem bleeding*

In some European reports, the sole cause of stem-bleeding symptoms which we consider similar to AOD was attributed to attack by *Agrilus*. Falck (1918) was one of the first to report the association of slime flux (stem bleeding) and *A. biguttatus* in declining oak in Germany. In the account he noted that: 'the occurrence of black slime flux was 'striking'; the seeping fluid was attributable to the feeding tunnels of the buprestid larvae which develop in living bark on both healthy, and more commonly, dying trees; the attacked trees can survive and recover; and only diseased stands suffered extensive attack'. Internal symptoms were not discussed in this paper, so it is unknown whether or not Falck (1918) knew about or recognized lesion formation in the inner bark of symptomatic trees. However, the stem-bleeding symptoms described by Falck (1918) do appear to be very similar to those we describe here, and this leads us to think that Falck (1918) was reporting a condition very similar to or the same as AOD.

Later, in 1950, a report on oak with similar stem bleed symptoms came from France (Jacquiot, 1950). It stated: 'on declined oak there was the presence of insect galleries running in the bark tissues and causing exudations of black liquid on the external surface of the rhytidome'. While in this sentence he attributes the stem bleeding to the presence of insect galleries further on in the manuscript he noted that the 'cambuim was necrotic' and stated that the tissue necrosis and abnormal tissue formation associated with callusing 'was a phenomenon of pathological origin' (Jacquiot, 1949, 1950). He went on to make two hypotheses: (1) That the insect served as a vector for a pathogenic agent – but Jacquiot (1949, 1950) dismissed this idea because he felt that the lesions were closely localized to the galleries and he reasoned that if necrosis was pathogenically induced, it would be expected to spread a long way in the tree tissues. (2) The second hypothesis suggested that the necrosis and abnormal tissue formation was a result of the larvae emitting a tissue-hypoplasia-inducing substance, which was so concentrated at the point of emission that it was toxic in the immediate vicinity, but further away it caused formation of abnormal (wavy) parenchyma. Again the symptoms described by this author are very similar to those for AOD, and we are more inclined to agree with Jacquiot's (1949) first hypothesis (see also Denman and Webber, 2009). Pathogenicity tests are required to prove the pathogenic agent hypothesis and chemical

ecology, and metabolomic studies are required to prove the alternate (second) hypothesis proposed by Jacquiot (1950). The research and interpretation of the situation presented by Jacquiot (1949, 1950, 1976) does, however, separate out three issues regarding symptomology: (1) tissue necrosis and the cause of this, (2) stem bleeding and the cause of this and (3) the formation of wavy parenchyma tissue and the cause of this. These three issues must be resolved to make headway in understanding the aetiology of the symptoms of AOD; and the fourth issue, signs of *A. biguttatus*, must also be understood (see below). However, based on the description given by Jacquiot (1949, 1950, 1976), it would seem that the condition he described on pedunculate oak in France is the same as AOD in Britain.

Forty to fifty years after reports by Jacquiot (1949, 1950), Hartmann and Blank (1992) and Van Steenkiste *et al*., (2004) from Germany and Belgium, respectively, referred to oak with symptoms similar to AOD (see Table 2) and attributed the stem bleeds to the activity of larval hatchlings of *A. biguttatus*, which tunnelled into the phloem tissue of stems and established a feeding nursery. They maintained that the tree responded to the tunnelling activity by producing dark fluid. This is in agreement with Falck (1918) and Jacquiot (1950). However, they (Hartmann *et al*., 1989; Hartmann and Blank, 1992, 1993) explained that the slime flux or bleed response only occurred on vigorous trees enabling them to overcome the attack and was thus probably a defence mechanism to flush the feeding larvae from the tree.

We interpret the role of *Agrilus* in AOD in Britain differently from these authorities. Although it is tricky determining which species of beetle larvae form the galleries from visual inspection alone, we used sequencing of the CO1 (cytochrome C oxidase 1) gene region to confirm the identity of larvae obtained from one site (Brown, 2014) and since the galleries are so uniform in appearance, we based our interpretation on this result. Furthermore, we have once witnessed larvae close to a bleed region in an AOD-symptomatic tree (Figure 12). Thus, we agree with the European reports that the galleries are caused by *A. biguttatus*. Our research showed a co-occurrence of galleries of *A. biguttatus* larval feeding and stem bleeds (>90 per cent) only evident upon destructive sampling, but we did not observe that the weeping patches were restricted to vigorous trees, rather that they occurred on trees of varying vigour (based on the condition of the crown of the tree). This is different to the idea proposed by Hartmann and Blank (1992, 1993). We also noted that active bleeding occurred long after *Agrilus* colonization had ceased and often lignified-callus-like tissue was growing over gallery damage. It is energy expensive for an oak tree to continue weeping fluid in response to larval feeding long after the event has taken place, arguing against the idea that *Agrilus* larval attack causes stem bleeding, unless it attracts further *Agrilus* attack but we did not evidence this. Furthermore, in some of our studies on other oak diseases such as *Armillaria*, we noticed that *Agrilus* colonization occurred or was underway on trees primarily attacked by root pathogens, but no bleeds or weeping stem patches were present on the stems that were colonized by *Agrilus*. This observation suggests that *Agrilus* attack does not induce stem bleeding or at least does not always induce stem bleeding. Finally, weeping patches occurred on a small minority of trees where there was no evidence at all of *A. biguttatus* attack suggesting that stem bleeding was caused by another agent. Our observations thus challenge the idea that stem bleeds in AOD (or similar based on the European reports) are caused solely by *A. biguttatus*. In further support of this, it was noted in Poland that stands suffering severe attack by *A. biguttatus* did not show symptoms of stem bleeding or 'slime-flux' (Hilszczanski and Sierpinski, 2006); however, it was interesting to note that 'even young trees (30–40 years old) were attacked by *A. biguttatus*'. Additionally, in a recent study carried out in Germany, (Kätzel *et al*., 2010) pointed out 'that only in some cases, were bleeding spots associated with points of attack by *Agrilus* species, on weakened oak'. Thus, while we acknowledge the explanation for the apparent absence of *Agrilus* larvae through mortality caused by the bleed

Table 2 Host species showing AOD or similar symptoms, country of occurrence and reference

Country	Scientific name	Common name	
Austria	*Quercus robur*	Pedunculate oak	Donaubauer, 1987; Cech and Tomiczek, 1986
	Q. petraea	Sessile oak	
Belgium	*Q. robur*	Pedunculate oak	Van Steenkiste *et al*., 2004
	Q. petraea	Sessile oak	
France	*Q. robur*	Pedunculate oak	Jacquiot, 1949; Jacquiot, 1950; Jacquiot, 1976
	Q. petraea	Sessile oak	
Germany	*Q. robur*	Pedunculate oak	Falck, 1918; Krahl-Urban and Schwertfeger, 1944; Hartmann *et al*., 1989; Hartmann and Blank, 1992; Kehr and Wulf, 1993; Schlag, 1994
Italy	*Q. cerris*	Turkey oak	Scortichini *et al*., 1997
Netherlands	*Q. robur*	Pedunculate oak	Oosterbaan, 1990; Oosterbaan and Nabuurs, 1991
Poland	*Q. robur*	Pedunculate oak	Siwecki, 1989; Kowalski, 1996; Siwecki and Ufnalski, 1998
Spain	*Q. ilex*	Holm oak	Soria *et al*., 1997; Biosca *et al*., 2003; Poza-Carrion *et al*., 2008
	Q. pyrenaica	Pyrenean oak	
UK	*Q. robur*	Pedunculate oak	Gibbs and Greig, 1997; Gibbs, 1999; Denman and Webber, 2009; S. Denman (unpublished)
	Q. petraea	Sessile oak	
	Q. cerris	Turkey oak	S. Denman (unpublished)
	Q. fabri	Bai li	S. Denman (unpublished)

response (i.e. hatchlings/larvae either drowned or exuded by fluid production) and have even on one occasion seen a number of medium-sized-to-mature larvae that appeared to have drowned in the fluid of lesions that developed close to the larval feeding galleries, we feel that this only applies to a very small minority of cases (Figure 12). Therefore, although the explanations on causes of the weeping patches offered by Hartmann *et al*. (1989), Hartmann and Blank (1992, 1993) Schlag (1994), Moraal and Hilszczanski (2000) and Van Steenkiste *et al*. (2004) are rational, well thought through and elegant in inception, it remains to be proven that *A. biguttatus* larvae do indeed induce stem weeping in oak. Similarly, experimental evidence explaining why stem bleeding does not occur in all *Agrilus* attacks is also required. However, since current evidence in Britain demonstrates that stem bleeding is not solely associated with *Agrilus* attack on oak, but that stem bleeding is a diagnostic and consistent symptom of AOD (i.e. always present), and there is a high co-occurrence of *Agrilus* with inner bark necroses, a key issue is to determine whether this is merely a co-incidental relationship or an essential part of AOD. As mentioned above, the actual cause of the stem bleeding in AOD must also be resolved.

Bacterial stem bleeding

There are only relatively few reports of bacterial diseases and bleeding canker on oak trees. One of the first reports of bacteria on oak was of *Erwinia quercina* Hildebrand and Schroth (Hildebrand and Schroth, 1967) causing gummosis of acorns in *Q. kelloggii* Newb. and *Q. wislizenni* A. DC., but with no mention of bleeding cankers. This species is now considered a subspecies of *Lonsdalea*, *Lonsdalea quercina* subsp. *quercina* (Brady *et al*., 2012). Convincing first evidence of bacterial-induced stem bleeding, canker formation and necrogenic activity in the inner bark of holm oak (*Q. ilex* L.) in Spain was provided by Soria *et al*. (1997) where the necrogenic activity of *L. quercina* ssp. *quercina* sp. nov. (*Lqq*) on holm oak was demonstrated. Although the disorder in Spain occurs on holm oak (see Table 2), this finding is consistent with the idea that bacteria may play a role in causing the stem bleeding on pedunculate and sessile oak affected by AOD in Britain as suggested by Denman and Webber (2009) and Denman *et al*. (2010) and work is underway in this regard (Denman *et al*., 2013).

The above section of the discussion has thus pointed out that various agents may induce stem bleeding on oak, but discerning inspection usually distinguishes the different forms of stem bleeding caused by these agents, and if there is any doubt, laboratory testing should be carried out. It has also highlighted that there are a number of views on the causes of stem-bleeding symptoms similar to AOD, but the cause of the bleeding has yet to be proven experimentally.

Diagnostic symptom (2): cracks/fissures between bark plates

A second diagnostic feature for AOD is cracking in the outer bark. Cracks may be caused by a number of agents for example, drought and frost (Hartmann *et al*., 1989; Hartmann and Blank, 1992). Cracks caused by these agents are compared with AOD cracks below.

Frost cracks

Frost cracks are initiated in the wood of the tree and so are very different to the bark cracks in AOD because they are much deeper. Also, frost cracks are generally rare in the UK owing to relatively mild winters, but they have been reported damaging oak in continental Europe (Krahl-Urban *et al*., 1944; Oleksyn and Przybył, 1987; Hartmann *et al*., 1989). Frost cracks differ from AOD cracks because: (a) there is no fluid seepage (bleeding) associated with frost cracks, and (b) frost cracks are generally much longer (2–15 m) (Hartmann and Blank, 1992) than those typifying AOD (2–20 cm). (c) The onset of occlusion to repair damage by frost cracks is immediate (Donaubauer, 1987) whereas it takes a few years before AOD lesions show signs of occlusion. (d) Frost cracks also mostly appear on the southern and western sides of trees where sunny winter days warm the bark of the exposed side followed by extreme temperature drop during night frosts, which kills the underlying bark tissues. AOD cracks can occur on any side of the tree, and although there is often a preference for the warmer sides of the tree (south side), aspect is not a diagnostic feature for AOD. (e) Sessile oak is more susceptible to frost damage than pedunculate oak (Hartmann *et al*., 1989; Schlag, 1994), but both oak species are equally affected by AOD. We thus conclude that frost cracks are clearly different from AOD cracks.

Drought cracks

Although drought-induced cracking and stem cankers have been recorded on scarlet oak (*Q. coccinea*) in North America (True and Tryon, 1956), no similar reports of this on *Q. robur* or *Q. petraea* have been found.

Diagnostic symptom (3): inner bark and sapwood necroses

Tissue necroses underlying the bark cracks are also a key diagnostic and main feature of AOD. It was particularly notable that AOD lesions often seemed to be initiated or centred on rays. Many different agents cause bark necroses and reports of bark necroses on oak are numerous, so only those that have strong similarities to AOD necroses are discussed here, and these include inner bark necroses caused by frost damage, fungi, bacteria and *Agrilus*.

Inner bark necroses caused by frost

A number of authors describe phloem and cambial necroses caused by frost damage, up to several metres long on oak stems, and this corresponds well with the reported length of frost-induced bark cracking (see above) (Krahl-Urban *et al*., 1944; Oleksyn and Przybył, 1987; Hartmann *et al*., 1989). Following frost damage, the colour of the inner bark changes uniformly to tawny brown and as the affected tissues dry out, sheets of loose bark fall off the side of the tree (Hartmann *et al*., 1989; Hartmann and Blank, 1992). This has not been recorded for AOD; in contrast, AOD lesions are wet, organic in shape, expanding and varying in dimensions, with areas of live tissue interspersed between them. Developing AOD lesions also display different shades of dark-to-light brown discolouration bounded by dark zone lines, and fresh lesions appear to have leading edges (i.e. expanding margins or break-out zones) compared with the uniform discolouration that frost damage causes. In AOD, the lesions usually progress to

form cavities whereas in frost damage, secondary microbial agents, usually fungi, exploit the weakened tissues and cause secondary infections. The bark does not automatically fall away from AOD lesions but may do so if lignified-callus-like tissue is formed and the lesion occludes pushing outwards loosening the outer cork layer until it falls away, in which case only a small area of bark (few square centimetres) would be lost. We can conclude that bark necroses caused by frost damage are significantly different and distinguishable from AOD tissue necroses.

Inner bark necroses caused by fungi

Fungal necroses of the inner bark of oak may be similar to AOD lesions. Phloem lesions on 'middle-aged' and old oak (*Q. robur*) in southern Poland were reported by Kowalski (1991) who gave a detailed account of the fungi isolated from bark necroses. Two symptoms were described: First, 'a local necrotic area in bark and phloem' from which a range of fungi were isolated and no specific causal role in the necrosis attributed to any of them, and secondly 'extensive necrotic areas caused by a range of agents' including *Armillaria*, *Phellinus robustus* (P. Karst) Bourdot & Galzin and *Nectria* L.'. The photographs in the manuscript and descriptions of *local bark lesions* given by Kowalski (1991) are very similar to AOD necroses, but the conclusion reached was that the cause of the lesions had yet to be explained, i.e. fungal pathogenesis was not seen as the cause of the localized lesions. Based on the symptoms he described, it does seem probable, however, that Kowalski (1991) was working on trees with AOD or a very similar condition. In contrast, in the western part of Poland (Krotoszyn Plateau), Siwecki (1989) reported oak mortality associated with the presence of dark black spots on the tree trunk and frequent isolation of *Ceratocystis* spp. Again, the photographs in the text are characteristic of AOD, but the description of the symptoms is not clear enough to be sure that Siwecki (1989) was working on AOD. In any event, neither the species identity of the fungi nor their effects on oak tissues was resolved, suggesting the necrosis was not attributed to fungal activity.

Another well-documented and striking account of a similar condition described on both *Q. robur* and *Q. petraea* in Austria is given by Donaubauer (1987) (see Table 2) and Cech and Tomiczek (1986). These authorities mention first indications of its appearance in Austria in 1945, with an infrequent occurrence in contrast to the marked increase in its presence between 1971 and 1982; but 'the actual cause still remains unclear' (Donaubauer, 1987). The condition was referred to as the 'T-disease' but nowadays the name 'T-disease' is applied to various canker diseases on a range of tree species, in which the old cankers have become occluded and therefore look T-shaped when exposed in a stem cross section, and the name is thus generic rather than diagnostic for a specific causal agent.

In Germany, a study to link fungi with various symptoms of oak decline was carried out by Kehr and Wulf (1993). Symptoms described and a photograph of a cross-sectional stem disc of Tree 6 bear likeness to AOD, but the conclusion the authors arrived at was that there was no evidence to support a causal role for fungi in the stem necrosis although it could be assumed that *Pezicula cinnamomea* (DC) Sacc. was likely to play a role in lesion enlargement (Kehr and Wulf, 1993). Previous to this study, Hartmann *et al.* (1989) and Hartmann and Blank (1992) distinguished various stem lesion symptoms on oak and designated them Type A, B and C. Of the three, Type B most closely resembles AOD. Hartmann *et al.* (1989) thought that *P. cinnamomea*, *Cytospora intermedia* Sacc. and *Fusicoccum quercus* Oudem., which they considered weak Ascomycetous pathogens, may cause the lesions but were most likely only secondary. However, because pathogenicity was not proven, no final conclusions were made about the role of these fungi in causing necrosis.

Inner bark necrosis caused by bacteria

In Belgium, Van Steenkiste *et al.* (2004) regarded the inner bark necroses to be caused by secondary colonization by 'bacteria, viruses and fungi', but no experimental work was presented to prove this hypothesis. There is thus a parallel between Van Steenkiste *et al.* (2004) and Hartmann *et al.* (1989) in that both these authorities suggest that the necroses are secondary which in the case of AOD implies in the first instance that they follow *Agrilus* attack and in the second instance that they may have multiple, random, but site-dependent causal agents.

Bark necroses on Mediterranean oak species were reported by Biosca *et al.*, (2003) who showed *Lqq* induced necrosis causing stem cankers and acorn gummosis on holm oak and Pyrenean oak (*Q. pyrenacia* L.) in Spain and corroborated findings by Soria *et al.* (1997). Collaboration between Spanish and British researchers is currently exploring the possibilities of parallels between the situation in Spain and Britain (Denman, personal communication). A report of bacteria on declining turkey oak (*Q. cerris* L.) with symptoms of stem bleeding in Italy was made by Scortichini *et al.* (1993). They showed that *Pantoea agglomerans* (Beijerinck, 1888) Gavini *et al.*, (1989) [previously *Erwinia herbicola* (Lohnis, 1911) Dye, 1964] was predominant in healthy as well as necrotic tissue of *Q. cerris*. However, pathogenicity tests yielded negative results, and none of the 98 bacterial strains (taxa) isolated from the trees were considered responsible for the decline.

In Britain, bacterial species have been isolated consistently from oak (*Q. robur* and *Q. petraea*) with symptoms of AOD (Denman and Webber, 2009; Brady *et al.*, 2010; Denman *et al.*, 2012). Two of these species, *Brenneria goodwinii* and *Gibbsiella quercinecans*, were isolated consistently from the necrotic margins of lesions in AOD-symptomatic trees (Denman *et al.*, 2013) but have also been isolated from mature AOD-symptomatic *Q. cerris* at two separate sites in the UK (see Table 2) (S. Denman, unpublished data) suggesting a possible causal role in lesion formation in AOD. Studies investigating the necrogenic activity of these taxa are underway.

On the other hand, Jacquiot (1950) provides a counter argument to the involvement of bacteria in necrosis of oak inner bark. He stated that five bacterial species were isolated from pedunculate oak with stem-bleeding symptoms (unfortunately the bacterial species were not formally named), but 'all the species of bacteria were inoculated on cultures of cambial tissue of *Quercus pedunculata*, on which they produced no effect'. Thus, he appears to reject the idea of a necrogenic role for bacteria (Jacquiot, 1950).

Inner bark necroses caused by Agrilus

While studies investigating involvement of bacteria in necrosis of the inner bark in AOD are currently underway, it is important not to forget the claims that *Agrilus* can cause lesions (e.g. Jacquiot, 1950). Although there is a high co-occurrence in the presence of larval galleries of *Agrilus* and inner bark necrosis in AOD, we

maintain that larvae are unable to cause tissue necrosis – they gnaw and ingest bark creating sinuous galleries, and this activity cannot rot tissue, i.e. cause spreading necrosis. Jacquiot (1950) attributed the lesions to phytotoxic levels of stimulatory biochemical compounds in the larval bore dust (frass) of *A. biguttatus*. It was hypothesized that the substance was toxic in the immediate vicinity of the point of secretion, thereby causing necrosis, but that as it diffused through the inner bark, it became stimulatory causing the proliferation of cells (Jacquiot, 1950, 1976). This must still be proven experimentally in oak tissues and does not fully account for complete degradation of tissue leading to cavities or for the spreading nature of the lesions and does not explain why most of the galleries in trees affected with AOD have sharply defined edges covering quite large distances (1–1.5 m) in the tree whereas the necrosis is laterally more extensive often far beyond or away from galleries.

From the above-mentioned studies, it is clear that the lack of detailed symptomology has hampered recognition of 'like' conditions. However, we can conclude that in the above-mentioned studies on pedunculate oak showing symptoms very similar to AOD, the cause of tissue necrosis was not proven for any particular fungal species. A role for bacteria in causing lesions seems possible based on research carried out on *Q. ilex* and *Q. pyrenaica* in Spain and on preliminary results from Britain. In recent findings in Britain, bacteria have also been isolated from turkey oak (*Q. cerris*) and Bai li oak (*Q. fabri)* with typical AOD symptoms (S. Denman unpublished, see Table 2) so it seems that these oak species are also susceptible to AOD but testing must confirm this.

Diagnostic symptom (4): larval galleries of Agrilus biguttatus

Signs of *Agrilus* larval activity in association with AOD have been discussed in some detail above, but we reiterate that external signs of *Agrilus* attack (i.e. exit holes) only occur on a small portion of AOD trees (30–35 per cent) whereas internally *Agrilus* galleries are found in >90 per cent of the trees studied. It is worthwhile mentioning that the D-shaped exit holes of *A. biguttatus* (Figure 5) may be confused with the slightly larger oval exit holes of the adult long-horned beetles (*Cerambycidae*), see Figure 19, but careful inspection and measurement of the longest part of the exit hole can help avoid this misinterpretation. The proportion of trees we recorded with external signs of *Agrilus* is corroborated by Brown (2014) who explains that this may be indicative of many larvae not completing their life cycle successfully in AOD attacks. The ecological and etiological implications of this need to be considered, but it seems unlikely that it would be an advantageous survival strategy for the beetle to develop but would benefit the attacked oak showing that they were able to overcome colonization. Thus, we conclude that the role of *Agrilus* in AOD has yet to be resolved and fully understood in terms of the ecological landscape.

Other aspects of symptom development

Effects of callusing on AOD development and tree health

It is unknown whether the occlusion of lesions completely halts their development or whether it is merely a form of remission and the occluded lesions will relapse, with necrosis reactivating in the new phloem laid over the lignified-callus-like tissue. Furthermore, there is evidence to show that even if lesion occlusion has occurred, reinfection can take place in new inner bark. Both these possibilities have been evidenced in some of the trees we studied, and this is an important research gap that requires addressing if we are to develop an understanding of the full impact of AOD on native oak populations. Long-term monitoring and dendrochronology studies are essential to address this and will give insights into the temporal development of AOD.

Links with root condition

In this study, we had a cursory look at root condition in 5 of the 21 trees to see whether there was any obvious connection with root rot (data not shown). No visual evidence of serious root damage as a consistent factor associated with AOD was seen, and there was no obvious link between the stem bleeds and root health. However, the root study was very limited and more research is required to determine the impact of root health on AOD in terms of predisposition and mortality of affected trees, as tree death is likely to be caused by multiple factors not AOD alone, and root condition will probably play a key role in tree survival. Thus, we consider root condition and the soil environment as major elements still requiring a research effort to determine their significance in AOD.

Estimation of appearance of AOD in Britain

In this paper, the defining symptoms of AOD are described in detail and each component discussed in the context of apparently similar conditions on pedunculate and sessile oak in Europe. Other authorities appear to have recognized a similar condition but have not identified it as a distinctive disorder within the overall oak decline syndrome. By considering the historical accounts of these similar conditions, the first of which was observed in the early 1900s in Germany (Falck, 1918), we can estimate the likely arrival/emergence of AOD in Britain. Using one of the European arguments that *Agrilus* is considered the cause of one of the key symptoms (i.e. stem bleeds) and based on the fact that this buprestid is central to AOD and European reports on oak showing similar symptoms, we can explore the temporal occurrence of AOD in relation to *Agrilus* in the UK. *A. biguttatus* is considered native to Europe (Bílý, 1982) including the UK (Levey, 1977), and because the weeping patches are so eye-catching, it seems unlikely that previous reports about the beetle would have overlooked this feature associated with its presence. The earliest reports of the beetle in Britain are in the 1880s (Fowler, 1888), but it was almost a hundred years later in the 1980s and 1990s that pathologists, Gibbs and Greig (1997) and Foster (1987), made the connection between *Agrilus* and stem bleeding in Britain. The eminent forest pathologist Dr W.R. Day also worked on oak decline specifically, in the 1920s and 1930s (Day, 1927, 1938), but made no mention of the stem-bleeding symptoms or of *Agrilus* damage. This suggests that the condition was not present in Britain at that time. In the 1950s, Dr Tom Peace, Head of Pathology at Forest Research, Alice Holt, Farnham, and Dr Bletchley, Head of Entomology at Forest Products Research Institute, Princes Risborough, stated that no reports of an attack of this kind (i.e. AOD) had been made to them (footnotes: Jacquiot, 1950). AOD was first noticed in Britain around the 1980s (Gibbs and Greig, 1997; Goodwin, 2005; David Rose, DDAS, FR; Brian Greig, Arboricultural Advisory and Information Services; Peter Goodwin (Woodland Heritage) personal communication).

In the wider European region, similar symptom descriptions on declined oak were previously reported from Belgium (Van Steenkiste *et al*., 2004), Germany (Falck, 1918; Hartmann *et al*., 1989), Poland (Kowalski, 1990, 1996); Netherlands (Oosterbaan, 1990), Italy (Vannini and Luisi, 1990; Scortichini *et al*., 1993) and Spain (Biosca *et al*., 2003) (see Table 2). Thus, it is probable that AOD also occurs in continental Europe and that some of the causal agents may even have spread from there to Britain, where symptoms were first noticed only ~30 years ago. In our studies in Britain, *Agrilus* is present in almost all cases researched so far, but the nature of its presence and possible interaction with other putative causal organisms is still under study.

Final comments and conclusions

We have set a baseline for the recognition of AOD in Britain, but our overall conclusion is that more research is required to understand the full nature of AOD. The precise sequence of events causing stem symptoms has yet to be proven, as have the causes of tree death. It is likely that the stem bleeding and bark necroses follow other predisposing factors, which will probably include soil factors such as poor fertility, drainage, moisture holding capacity or compaction and may include a role for the rhizosphere microbiota. Based on interpretation of symptomology in some of the AOD cases, where larval damage occurred in the cambial/sapwood layers prior to the development of callus-like tissue and lesion formation took place in the phloem formed over this tissue, it is tempting to think that *Agrilus* colonization occurred prior to the development of symptoms (Figure 20). This view has also been expressed by Van Steenkiste *et al*. (2004) but Brown (2014) recorded the presence of stem bleeds before the occurrence of the exit holes of *Agrilus*, although galleries were present in most of the trees showing stem bleeding. Clearly there is still much work to be done on interpreting and understanding the epidemiology of AOD and the sequence of events.

Results of this study show that most sites were on clay-rich, slowly permeable, seasonally water-logged soils, so soil type and drainage may be a site factor involved in predisposition of the trees. The genetic potential of the trees, host age and climate change effects should also be considered. Our research initiative thus works to the decline spiral model depicted by Manion and Lachance (1992). We are also using post-genomic tools to obtain insights into the causes of AOD and roles that the different biotic agents play in the syndrome.

In this paper, we have described the symptoms and elucidated the key diagnostic features of AOD thereby providing a baseline from which to identify and compare similar disorders. However, the unmistakeable symptoms of AOD also lead us to hypothesize that this is a distinctive condition currently identified on oak (but could possibly include other host species as well). Further host range and pathogenicity studies are required to prove this hypothesis, and the damaging effects of AOD on tree function must be determined. To estimate the long-term effects of AOD on Britain's native oak species, it is essential to know the distribution and rate of spread of the condition in Britain, mortality levels, effects of remission, as well as the environmental and host conditions necessary for this decline-disease to take hold. Once this information is in hand, appropriate management strategies can be considered.

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Conflict of interest statement

None declared.

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