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The ash wilt disease: a preliminary investigation of wood anatomy

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Abstract

A macroscopical, microscopical and SEM examination of wood samples, taken from diseased ash trees, was undertaken to probe into the cause of an ash wilt disease considered to be identical with dieback. The symptoms, particularly the obstruction of the vessels by tyloses and the occurrence of fungal hyphae in one specimen, bear a strong resemblance to those found in Dutch elm disease.

Additional keywords: Dieback, *Fraxinus excelsior*, hyphae, tyloses, parenchymatous cells, SEM.

Introduction

The decline of ash, particularly *Fraxinus americana* L. (white ash) in the United States of America and *F. excelsior* L. (common ash) in Great Britain, has become increasingly widespread since the 1950's (Pawsey, 1973; Hibben and Silverborg, 1978). In their definition of this disease, Matteoni and Sinclair (1985) were careful in their application of the name 'ash dieback' and used this term as meaning abnormal mortality of twigs, branches and trunks. They referred to the term decline as meaning a gradual failure of health. The most common name in use for this disease, however, is dieback.

Although Silverborg and Brandt (1957) suggested *Cytophoma pruinosa* (Fries) Höhn. as the primary cause of ash dieback, they also considered the possibility that this fungus may only be a secondary pathogen. Many authors referred to a concept of multiple causes (Hibben and Silverborg, 1978; Matteoni and Sinclair, 1985). Matteoni and Sinclair (1985), however, were convinced that mycoplasma-like organisms must be assigned an important role. Tobiessen and Buchsbaum (1976) and particularly Ross (1966) considered a period of drought as significant for the initiation of the disease. This view was partially shared by Hibben and Silverborg (1978) who also took other possibilities into consideration such as air pollution, leafspot-causing fungi and viruses as well as another as yet unknown infectious agent.

In recent years a similar problem has arisen with *F. excelsior* in the Netherlands. In 1986, a relatively dry year, the problem became worse. Although it is not yet certain whether we are in fact dealing with the same disease, many symptoms that are being

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encountered relate with those described by the previously mentioned authors. The most consistent symptom reported, however, is that of wilting, although sunken patches along the tree trunks are frequently found. A thorough investigation is now in progress to describe the disease symptoms more completely. In the meantime, in order to minimize confusion, this problem in ash will be referred to as 'ash wilt disease'.

As wilting is usually associated with a disturbance of the sap flow, it was considered necessary to make a preliminary examination of the secondary xylem. In spite of many claims, no causative organism has as yet been proven responsible for the ash wilt disease. Any abnormalities found in this investigation, therefore, may help to gain a better insight into the actual cause or causal complex.

Material and methods

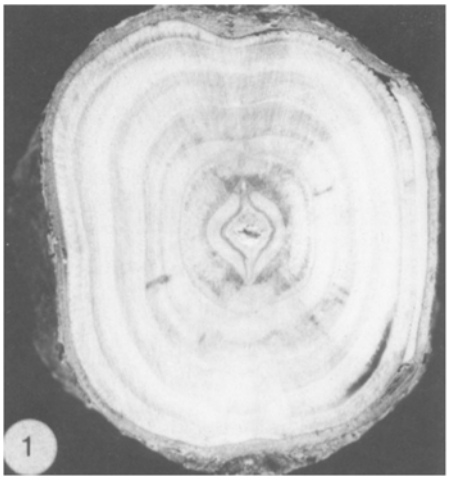
Samples were selected from 2-cm-thick discs of wood taken at a height of 1.3 m and 4 m from three freshly felled ash trees of approximately 10 years of age: (1) a tree with a crown of which one part was dead and another part was completely wilted; the bark was sunken, the remaining tissue of which was adjacent to the wilted part of the crown; (2) a tree dying from severe wilting; and (3) a healthy tree.

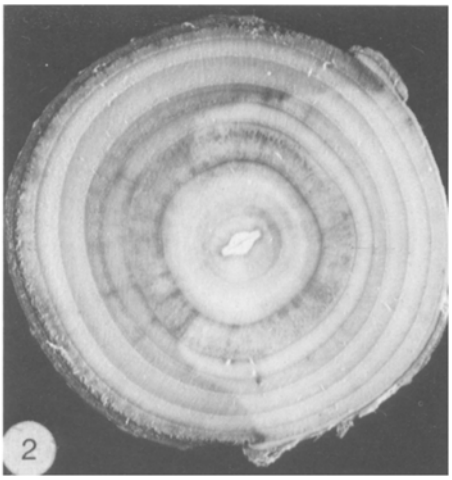
Transverse, tangential and radial sections 15 μm thick were made and stained with Fast Green for light microscopical examination. For scanning electron microscopy (SEM), small pieces of wood were selected and cut to expose a radial surface approximately 6 $\times$ 8 mm. After air drying the specimens were mounted on stubs, sputterd with platinum and examined with a Jeol JSM-35C scanning electron microscope.

Results and discussion

No major differences in the wood anatomy of the specimens involved in this study were found when comparisons were made with available descriptions of the wood anatomy of ash, in particular *F. excelsior* (Brazier and Franklin, 1961; Grosser, 1977; Schweingruber, 1978). Macroscopical examination of the discs of wood taken from tree 1 (Figs 1 and 2) revealed considerable tylose activity denoted by the dark colouration of the vessels particularly in the early wood. In the wood samples from tree 2 (Fig. 3) this phenomenon appeared to be limited to small areas of the last growth rings. Wood samples from the healthy tree (not shown) were free from any discolouration and tyloses were not found upon microscopical examination. On further microscopical examination of the 'diseased' wood, in particular from tree 1, tyloses were most frequently found in the early wood but were also often present in the late wood (Fig. 4). In the latter the vessels often contained granulated deposits (Fig. 5). The occurrence of tyloses in secondary wood, however, was reported to be a common feature, but mostly in the heartwood (Brazier and Franklin, 1961). Jane (1970) stated too that in adult wood a few early vessels containing thin-walled tyloses are not uncommon in ash. As the wood samples used in this investigation were taken from trees not older than 10 years the wood should be regarded as being in a juvenile state (Carlquist, 1962). Under these circumstances, therefore, the presence of tyloses must be considered abnormal. Their presence is highly suggestive of a reaction mechanism due to stress or other situations, e.g. the presence of a pathogen (Elgersma, 1973; Panshin and De Zeeuw, 1980).

Examination of the sunken area of the trunk of tree 1 revealed that a large percen-





Figs 1 and 2. Wood specimens from diseased tree no. 1, at 4 m and 1.3 m, respectively. Tylose activity is indicated by dark vessels. Note large coloured zones of wood which had ceased to develop further during 1985. Actual size.

tage of the secondary wood growth had ceased to develop during the summer of 1985. At 4 m and 1.3 m height, only 25% and 35%, respectively, of the growth ring had continued its growth until the tree was felled in 1986 (Figs 1 and 2). Microscopical examination of this area revealed a disturbance in the late wood of 1985 (Fig. 6) followed by a continuation of normal wood development. Characteristic for this zone were the large groups of parenchymatous cells, some of which may be pith flecks (Panshin and

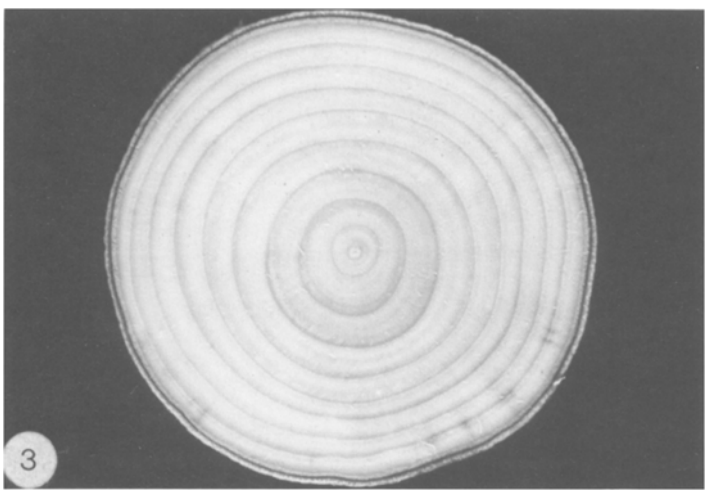
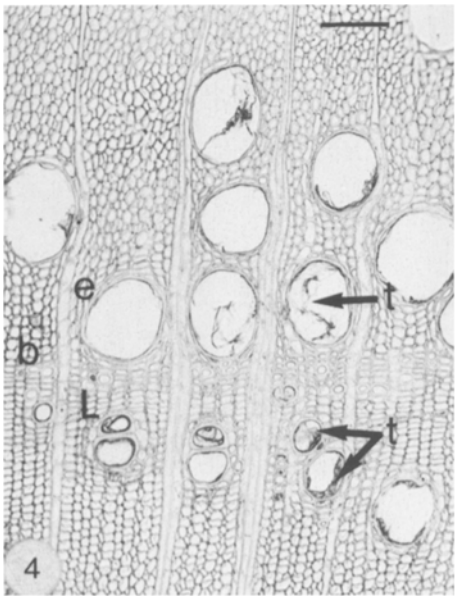
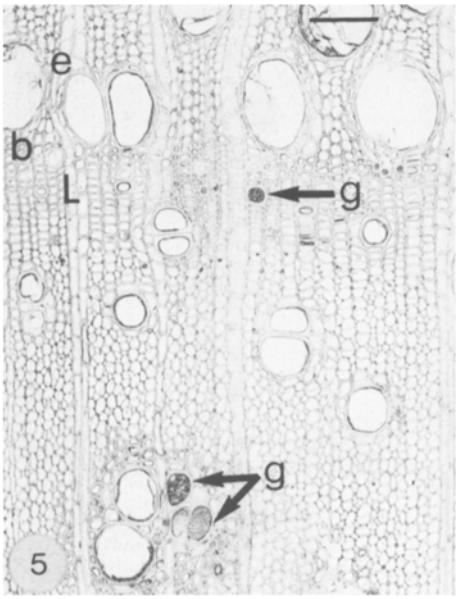
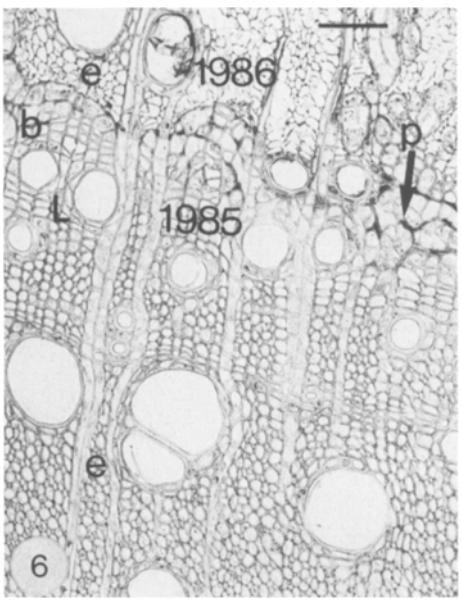


Fig. 3. Wood specimen from diseased tree no. 2, at 1.3 m. Dark vessels are mostly limited to the outer growth rings. Seventy-seven % of actual size.







Figs 4-6. Transverse sections from tree no. 1; (b) is border between late wood (L) and the early wood (e) of the next growth ring; bar represents 100 μm.

Fig. 4. Tyloses (t) in wood vessels of diseased ash.

Fig. 5. Late wood vessels with granular deposits (g).

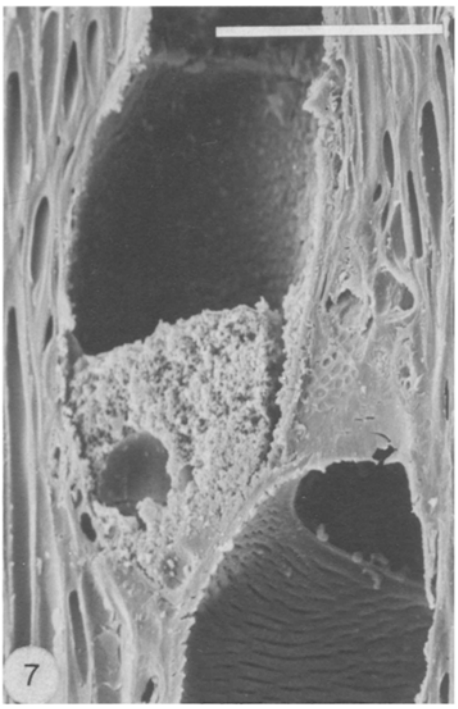
Fig. 6. A disturbance in wood development. Numerous groups of parenchymatous cells (p), some of which appear to form a barrier between the late wood of 1985 and the early wood of 1986.

Figs 7-11. SEM observations of radial surfaces; bar represents 100 μm. →

Fig. 7. Granulated deposits found blocking a wood vessel in tree no. 1.

Fig. 8. Thin walls of tyloses which have collapsed within early vessels of tree no. 2.

Fig. 9. Obstruction of a wood vessel by thick-walled tyloses in tree no. 1.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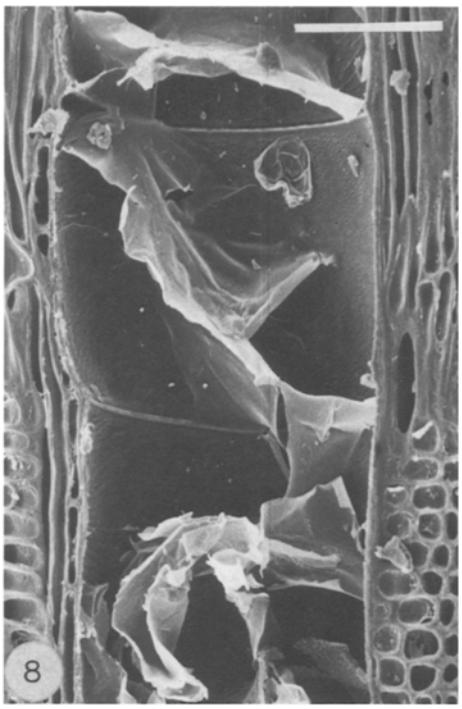
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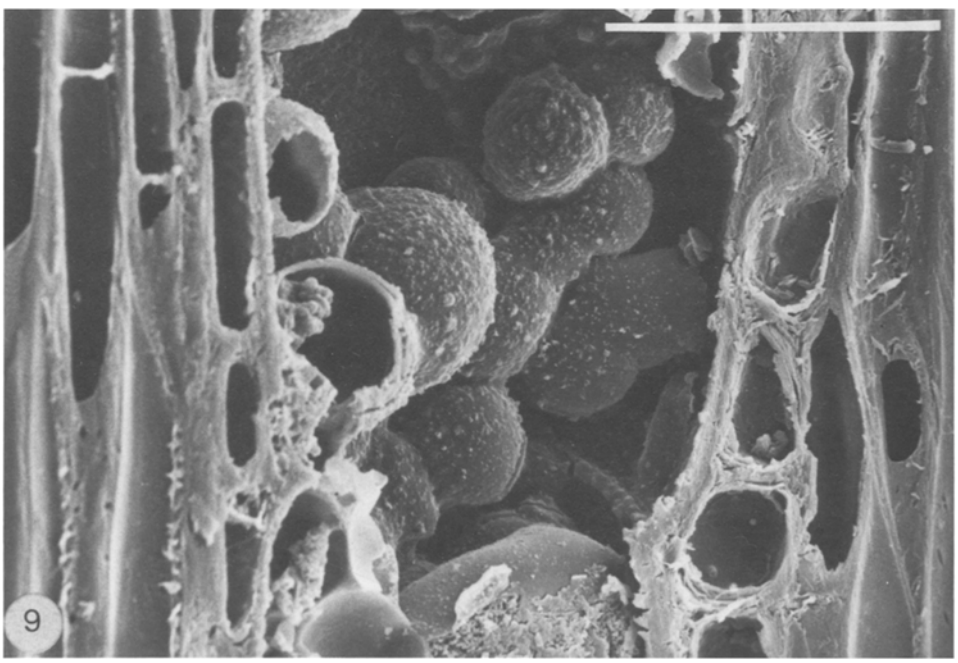
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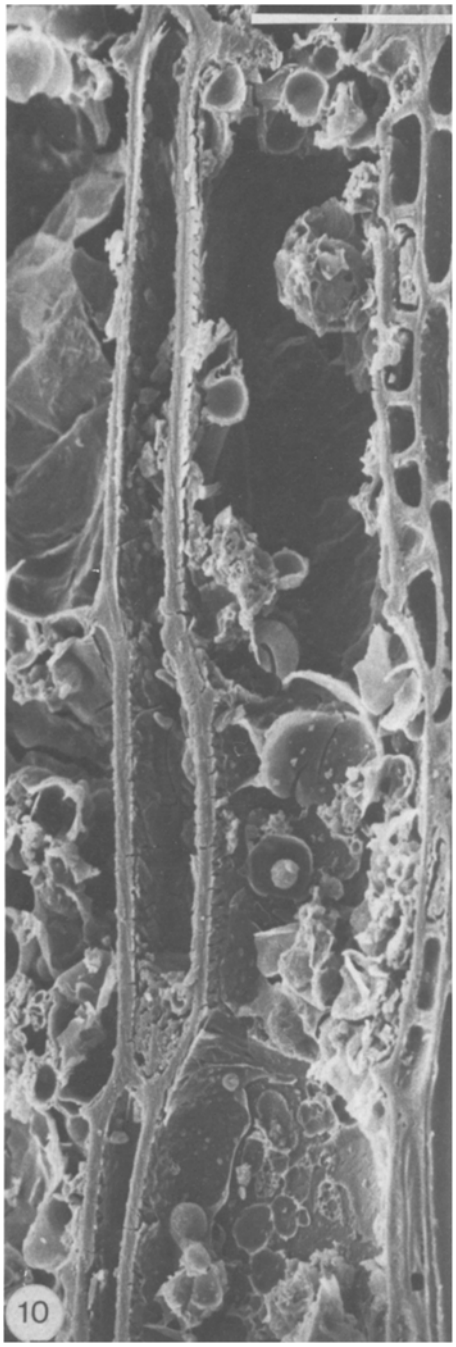

Fig. 10. Broken and collapsed, thick- and thin-walled tyloses as well as debris in the wood vessels of tree no. 1. An image very similar to that found in the Dutch elm disease.
Fig. 11. Fungal hyphae and spores found in a wood vessel of tree no. 2 morphologically resembling *Ceratocystis* or *Ophiostoma* species.

De Zeeuw, 1980) common in birch and maple but not reported in ash. Another explanation for the presence of parenchymatous cells is that these cells function as a defence barrier which prohibits the infection, already present in the 1985 growth ring, from further radial development (Moore, 1978; Shigo, 1984). Although the last hypothesis is plausable further studies are required to substantiate such a claim in this case.

Zonal colouration of wood samples, clearly seen in Fig. 2, appears in the sector where radial growth ceased in 1985. The cause is not known but dead or dying tissue of ash can be stained by certain fungi (Hibben and Silverborg, 1978).

The observations obtained by light microscopical examination could be verified by SEM. The granulated contents, often found in the vessels of wood samples from tree 1, were clearly demonstrated (Fig. 7) as were the thin-walled tyloses found in a few of the early wood vessels in samples from tree 2 (Fig. 8). In the last specimen only one late wood vessel was found by SEM to contain tyloses with granulated deposits. SEM examination of specimens from tree 1 revealed tyloses (Figs 9 and 10) that bore a resemblance to those found in Dutch elm disease (Miller and Elgersma, 1976). In the specimens from tree 2, one early vessel of the last growth ring was found to contain fungal hyphae with spores (Fig. 11) morphologically similar to *Ophiostoma* of *Ceratocystis* spp. (D.M. Elgersma, personal communication). With reference to this last observation it should be stated, however, that a fungal pathogen has not yet been isolated.

The problem in ash in the Netherlands is similar in many ways to the problem described by the previous authors and it is most probable that water stress and drought situations are involved in the disease development. With regard to the examination of the secondary wood of ash, there does not appear to be any record of research on this subject relating to ash dieback as previously described. It therefore becomes a much more difficult task to relate the results of this investigation to the other observations. Should, however, a yet unknown infectious agent be involved, as is suggested for ash dieback by Hibben and Silverborg (1978), then it is of considerable interest to note that the results of this investigation bear a resemblance to the results previously obtained from research of the Dutch elm disease, caused by *Ophiostoma ulmi*, and oak wilt, caused by *Ceratocystis fagacearum*.

The isolation of such a pathogen, whether facultatively parasitic or otherwise, is a requisite before one should speculate further on the cause of 'ash wilt disease' in this country. Not only should a search for the causative organism be continued but a more intensive anatomical examination of diseased ash wood is necessary to understand the mechanisms involved in the tree's reaction to biological and physiological stress. Only then is it possible to come to terms with ash wilt disease.

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Samenvatting

De esseverwelkingsziekte: een verkennend houtonderzoek

Houtmonsters van zieke essen werden macroscopisch, microscopisch en met de SEM onderzocht om de oorzaak van de 'esseverwelkingsziekte' op te sporen. Deze ziekte, ook wel aangeduid met 'essensterven', vertoont grote overeenkomsten met de in de Verenigde Staten van Amerika en in Groot-Brittanië al langer bekende 'ash dieback'. De gevonden verschijnselen, in het bijzonder de verstopping van vaten door thyllen en het vóórkomen van schimmeldraden in een van de monsters, vertonen veel overeenkomsten met karakteristieke symptomen van de iepeziekte en van de eikeverwelking.

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