WOOD AND BARK ANATOMY OF YOUNG BEECH IN RELATION TO *CRYPTOCOCCUS* ATTACK[1]

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Abstract.--Within a sample of European beech, partial resistance to attack by the beech scale, *Cryptococcus fagisuga*, was associated with a smooth bark which had a regular, vertical pattern in its surface 'growth lines'. Such bark contained relatively little lignified outer parenchyma, and the main stone cell layer was strongly developed. The 'dimpling' symptom of infested stems was brought about by locally reduced and abnormal xylem development.

INTRODUCTION

The bark structure of beech, *Fagus* spp. is of a type unusual among temperate hardwoods, since even in old trees live parenchyma tissue is present just beneath the outer surface. It is this tissue from which the beech scale, *Cryptococcus fagisuga* Lind. obtains its food supply. The relationship between the anatomy of the host and feeding by the insect is of relevance to beech bark disease (BBD) for two reasons. First, if there are any anatomical factors which can influence the availability of nutritive parenchyma tissues, they may determine whether or not infestation can become severe enough for the fungal phase of BBD to develop (Lonsdale 1980a). Second, it is known that abnormalities of the host tissues can develop in response to feeding by the insect, and it is possible that these abnormalities may contribute to the pathological processes involved in BBD.

The effects, if any, of host anatomy on susceptibility to scale feeding have received little attention, but it appears from the work of Kunkel (1968) and Braun (1976/77) that infestation itself can affect bark structure to the extent that feeding may be enhanced or inhibited by different phases of the histological response to attacks. Enhancement seems to occur at least for a short time due to the formation of gall-like structures within the outer bark parenchyma, while inhibition occurs when the cells in and around this structure become necrotic (Kunkel *op. cit.*). The formation of a wound phellogen at some depth beneath the primary phellogen occurs in response to feeding damage (Braun 1976b) and may serve further to isolate the outer parenchyma, thus causing extension of necrosis. A type of gall formation in young trees and also necrosis were described over a hundred years ago by Hartig (1878, 1880).

Little information is available on the possibly variable influence on insect feeding of the anatomy of trees which have never been previously infested, although it has been observed by Ehrlich (1934) and by Kunkel (1968) that the stone cells in outer bark can act as a barrier to stylar penetration. It has also been observed by S. Fink (pers. comm.) that trees with rhytidome formation, exceptional in beech, are more or less resistant to attack. This applies particularly to the 'male' or rough-barked beech (Feucht 1910, Münch 1914). The findings of Wainhouse and Deeble (1980) indicated that different genetic strains of beech may vary in their susceptibility to attack, and it is also a common observation that very young trees seldom become heavily infested. It may be that these differences are at least in part related to anatomical factors. The external smoothness and apparent hardness of the bark have been regarded as factors in susceptibility to attack (Eckstein 1920, Bertelsmann 1913, Reichling 1920), and attention has been drawn in particular to the role of fissures in providing suitable sites for settling of the insects (Thomsen, Buchwald and Hauberg 1949, Anon 1956). Surface growth of lichens (Ehrlich 1934) or of algae (Houston, Parker and Lonsdale 1979) may also enhance the build-up of colonies, while the fungus *Ascodichaena rugosa* Butin can act as a physical barrier to feeding (Houston 1976, Houston *et al. op. cit.*).

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As far as the health of the tree is concerned, there is uncertainty over the importance of the structural changes in the bark as reported by Kunkel (1968 and Braun 1977). Both these authors considered that the deep necrosis of the type typical of BBD could occur from scale feeding alone, and Braun (*op. cit.*) suggested that *N. coccinea*, though often present, was not essential in the etiology of BBD. However, no evidence for this has been obtained in the absence of micro-organisms and so they cannot be ruled out as a factor in the development of deep bark necrosis.

Another type of abnormal anatomy of infested stems is 'dimpling' or 'pitting'. In this condition the surface of the stem bears depressions approximately one centimetre in diameter and several millimetres deep. The first report which seems to describe the symptom in its current recognised form came from Thomsen *et al.* (*op. cit.*) in Denmark. The nature of dimpling has received little attention, although Parker (1974) suggested that it is the result of callusing around the margins of sites where small bark necroses associated with scale feeding have extended as deeply as the vascular cambium.

The objects of the present studies were to compare the bark structure of trees with apparently different degress of susceptibility, and to describe some of the anatomical abnormalities caused by scale feeding.

MATERIALS AND METHODS

The bark surface was examined *in situ* before and after removal of algae and lichens. The characteristics noted were as follows: general smoothness, shape and disposition of lenticels and abundance, size and orientation of vertical 'growth'lines. ('Growth' lines appeared to be associated with tangential expansion of the phellem.)

The bark anatomy was examined by cutting 15µm-thick sections on a freezing microtome after an embedding procedure involving six hours' immersion in a 5% agar solution at 45°C Phloroglucinal/HCl and Sudan Black were used to stain lignified and suberised tissues respectively. Other stains used were acid fuchsin and safranin/light green. The characters recorded were the thicknesses of the different tissue layers and the extent and continuity of stone cell layers. For examination of 'dimples' the wood and/or bark in the affected zones were transversely sectioned at 8-12 µm after a softening procedure in a mixture of equal parts glycerol and ethanol (Jane 1956). Longitudinal sections were cut at 2 µm using glass 'Ralph' knives (Lindner and Richards 1978). The frequencies and sizes of different xylem cell types were measured within the dimpled zone.

RESULTS

The bark surface in relation to susceptibility.

This study was carried out on eight clones comprising the four most resistant and the four most susceptible clones present in a seed orchard experiment (D. Wainhouse pers. comm.). The trees were located at Hemsted Forest in Kent (23 years old) and Alice Holt Forest in Hampshire (20 years old). In order to minimise the presence of alterations induced by insect feeding, bark samples were taken from zones of stem beyond the limits of any heavy infestation. The replicate trees, numbering from four to ten per clone, showed much less variation within clones than between clones. In view of the subjective nature of the assessments and the small number of clones available, the apparent differences between clones in bark surface morphology are expressed here in the form of the following observational summary. The general bark surface (i.e. excluding lenticels and 'growth lines') varied considerably in smoothness, and trees in all the 'susceptible' clones were rough, while the trees in three of the four 'resistant' clones were smooth. One clone with almost total absence of infestation was exceptionally smooth. Variations in lenticel shape, size and abundance showed little association with resistance. The most consistent difference between resistant and susceptible clones was in the pattern of 'growth lines'. These lines were present on all trees except on some replicate trees in two of the resistant clones. On the resistant clones the vertical linearity of these lines was considerably more regular than on the susceptible clones.

Several trees in the above study showed a linear pattern of *C. fagisuga* colonies and this was associated with fissures in the epiphytic covering of algae and lichens. In these cases the epiphytic covering was apparently so thick and dense that the fissures, apparently produced by expansion of the stem, were more favoured as feeding sites than the general surface.

Internal bark structure in relation to susceptibility

The data were obtained from three series of trees: the eight clonal trees mentioned above,

ten 'resistant' and 'susceptible' trees in a progeny trial whose genetic origin was different but unspecified, and fifteen plantation trees which had been heavily infested by *C. fagisuga*.

There was considerable variation in the relative thicknesses of the alternating layers of stone cells and unlignified parenchyma. As with the bark surface features, replicated clonal trees showed much less variation in bark anatomy between individual trees than between clones. In particular, the shape and extent of the sclerenchyma surrounding the heads of the sclerotic phloem rays was characteristic for a given clone. Among both the clonal trees and the genetically unreplicated progeny trial trees, there was a relationship between apparent susceptibility and the structure of the outermost layers of parenchyma and stone cells. In most trees there were two or three tangentially oriented layers of lignified cells. The outermost of these usually appeared poorly defined and discontinuous in transverse section, while the next layer was usually more strongly defined and in some trees was continuous over many millimetres of stem circumference. In trees classed as 'resistant', the depth of unlignified parenchyma outside the well defined stone cell layer was either small compared with 'susceptible' trees, or the outermost lignified layer was strongly developed. A few susceptible trees were atypical in that they had a very limited depth of external parenchyma, but the 'strong' stone cell layer was less continuous and less well defined than in the more resistant trees. Examples of bark from a resistant and a susceptible tree are shown in Figure 1.

An index was devised which incorporated the measurements of parenchyma and stone cell layers as follows:

$$\frac{100\ (a + b)^2}{\%L1\ .\ \%L2\ (a + b + c)}$$

where, as shown in Figure 1, a+b and c are the mean depths in μm occupied by the unlignified and lignified cells respectively in the tissue external to the 'strong' stone cell layer, and %L1 and %L2 are percentage values representing the degree of lignification within the layers designated.

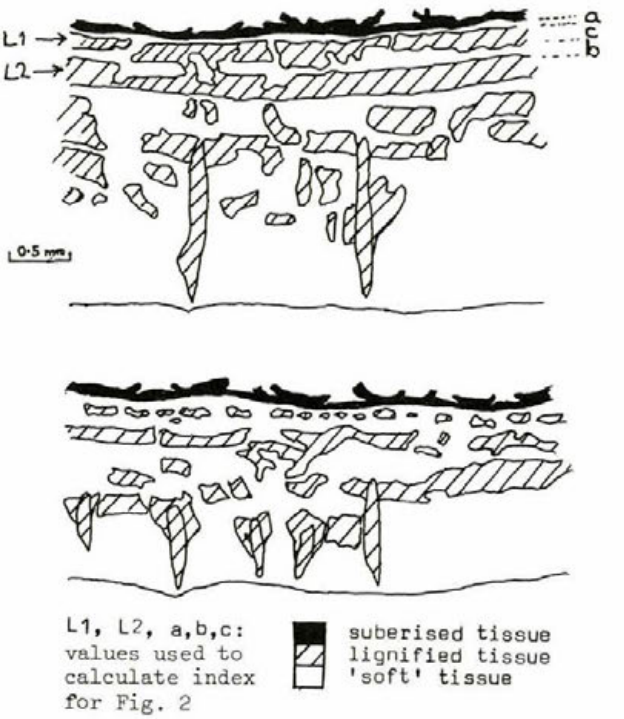

Figure 1.--Drawings of transverse sections of bark from trees with apparently high (above) and low (below) resistance to attack.

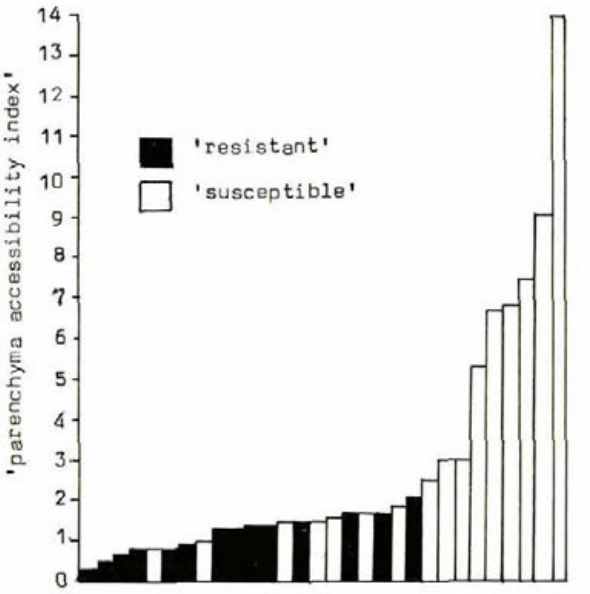

Figure 2.--Index values of the depth and accessibility of outer parenchyma in trees with apparent high and low resistance to *C. fagisuga* attack.

The evaluation of the index was carried out for bark samples from thirty trees and the results are shown in Figure 2. Replication of samples within trees was limited to between three and five 1 cm diameter bark discs, since it was established that variation within individual trees was small. The susceptible trees gave higher indices than the resistant trees. The most obvious exceptions to this were samples in which the tangential boundaries of the 'strong' stone cell layer were uneven; a score for unevenness was recorded for all samples but was not incorporated into the index.

Anatomical changes in bark due to *C. fagisuga* attack

Infested bark sections frequently showed gall formation and/or necrosis in the outer parenchyma and sometimes also in the parenchyma deeper than the 'strong' stone cell layer, the galls were 200-500 µm in diameter in the transverse plane. In addition to discrete galls, there was usually an abnormally thick phellem, suggesting stimulation of meristematic activity in the phellogen. Wound phellogens were frequently formed within the bark parenchyma. The gall tissues were often the sites of initial 'spot' necrosis, while old infestations were often associated with necrosis in much of the outer parenchyma. This necrosis affected the normal range of feeding depths for *C. fagisuga* so that heavy infestation could not have been maintained. Such bark was usually fissured however, and along the fissures, live parenchyma was present near the surface and hence accessible to *C. fagisuga*. These feeding sites were often the only areas of continuing infestation.

The nature of 'dimpling' or 'pitting'

Sections incorporating both bark and wood often showed that a necrotic spot was present in the bark parenchyma overlying a dimple. Such spots very rarely extended into the phloem and vascular cambium. Thus dimpling did not usually involve cambial necrosis. The wood showed a great reduction in annual ring width in the centre of a dimple, and most of this reduction was confined to one or two rings. The number of vessels in the intensely affected zone was very low, and often they were absent. The cells in general appeared undifferentiated and parenchyma-like. They tended to form chains of ray-like appearance. The number of cells across the ring width was also greatly reduced. Table 1 shows the results of a survey of intense dimples, and Plate 1 shows a transverse section of wood in which a dimple had formed nine to ten years before the sample was taken and where abnormal wood formation had continued and later re-intensified.

Table 1.--Dimpling : xylem cell size and number and ring width[a]

	Percentage of normal	
	Mean values for 26 dimples	Values for the most intense dimple
Total cell no.	36.3	7.8
Vessel no.	3.6	0.0[b]
Mean cell diameter	60.0	40.0
Ring width	24.9	3.3

[a] data relate to the most affected annual ring in each dimple, compared with values in the healthy zone of the same ring.

[b] of the 26 dimples, 22 had no vessels in the most affected zone.

Plate 1.--Transverse section of a 'dimple', showing abnormal and reduced xylem production.

DISCUSSION

The apparent relationships between bark structure, external and internal, and susceptibility to *C. fagisuga* attack seem relevant to recent studies on genetically determined resistance

(Wainhouse & Deeble 1980, Wainhouse, these Proc.). If these relationships are valid for a wider range of beech populations it may be possible to use them in developing selection procedures, both in the forest and in future planting programmes. Observations of six-year-old stems suggest that differences may appear at an early stage of growth (D. Lonsdale, unpublished data). If resistant trees can be anatomically characterised in this way, it may be possible to develop a workable method for the simple detection of the desired characters.

The time factor is extremely important in beech bark disease, not least with respect to the onset of susceptibility in *Cryptococcus* attack. Perhaps some of these temporal effects could be explained anatomically, and it is essential to take the time factor into account in any research which depends upon concepts of resistance and susceptibility.

The gall-like structures in the bark parenchyma were somewhat larger than the 'cell complexes' which Kunkel (1968) observed near the apices of individual insect stylets. They are, on the other hand, considerably smaller than the externally protuberant galls described by Hartig (1880). It seems likely, in any event, that they may enhance insect feeding, and, as such, are of interest in the population dynamics of *C. fagisuga*. Of similar interest is the necrotic breakdown of the feeding zones, noted previously by Kunkel (*op. cit.*) and by Braun (1977). It does not seem to have been noted previously that the fissures characteristic of superficially necrotic bark provide sites for residual *C. fagisuga* colonisation after much of the general bark surface has become resistant to attack by virtue of the necrotic layers. This must be set against previous statements (e.g. Anon 1956) that bark fissures are sites of initial, rather than residual, colonisation. This may be nearer the truth in the case of the 'fissures' in dense and deep epiphytic growth observed during the present studies. In either situation, the colonisation shows a 'line' pattern.

The nature of 'dimpling' or 'pitting' is of interest in relation to the observation that parenchyma feeders such as some adelgids and coccids produce plant hormone-like compounds (Balch, Clark and Bonga 1964). This has been suggested in the case of *C. fagisuga* (Fink and Braun 1980) and is a likely explanation for gall formation in the bark. However, the abnormal differentiation of vascular cambium occurs at some depth below the feeding zone, and the possibility must be considered that the effect may be an indirect one. In particular, the abnormalities are of a type often observed in cambial tissues exposed to ethylene, a gas which is produced by senescing tissue such as occurs in the necrotic feeding sites present in the bark overlying dimples. Similar defects have been artificially induced in beech wood by implanting the ethylene precursor 2, chloro-ethyl phosphonic acid into the bark (Lonsdale and Parker 1981).

Dimpling is known to occur on other tree species infested by scales, such as alder, sallow and ash (C. Carter, C.W.T. Young, D. Lonsdale, unpublished data), but detailed studies of xylem abnormalities seem to have been confined to conifers, such as *Abies* spp. attacked by Adelgids (Bryant 1974) and *Pinus pinaster* attacked by *Matsucoccus feytaudi* (Carle, Carde and Boulay 1970). The abnormalities in these conifers are different to those observed on beech in the present study but it is of interest that the abnormal production of short tracheids with narrow lumens seriously affects water movement in the xylem of *Abies* spp. (Bryant *op. cit.*). A beech tree with several years' heavy dimple formation might perhaps suffer loss of water conductivity, and this could contribute to the stress which seems prerequisite for *Nectria* attack (Houston 1973, Lonsdale 1980b). It is of interest in this connection that necrotic spots in the bark, associated with dimples, may harbour *N. coccinea* (Lonsdale and Sherriff, these proceedings).

The histological relationships between *C. fagisuga* and its host are relevant to stress and to latent infection by *Nectria* as well as to resistance mechanisms both preformed and elicited by insect attack. Perhaps studies of this type may help to explain the precise etiology of beech bark disease which remains uncertain.

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RÉSUMÉ

L'anatomie et la morphologie extérieure de l'écorce du hêtre furent étudiées en fonction des variations de sensibilité de différents clones d'arbres à l'infestation par la cochenille du hêtre, Cryptococcus fagisuga.

Extérieurement, l'ecorce des clones les plus résistants avait tendance à être plus lisse et montrait des 'lignes de croissance' soit peu nombreuses, soit ayant une forme régulière et verticale. A l'intérieur, les clones plus résistants différaient des clones sensibles par une épaisseur moindre et/ou une quantité plus grande de cellules lignifiées dans le parenchyme tendre situé à l'extérieur de la principale barrière de cellules scléreuses. C'est dans cette strate que l'insecte semblait s'alimenter et on propose l'hypothèse que l'alimentation n'a pas lieu facilement si la strate n'est pas arrivée à une certaine profondeur. Des observations analogues ont été faites sur des arbres dont l'origine génétique n'était pas connue.

L'anatomie du bois fut étudiée afin de déterminer la nature des lésions présentes sur les arbres envahis par le C. fagisuga. Les zones centrales des lésions contenaient un ou deux anneaux annuels dans lesquels le nombre de cellules de xylème était réduit considérablement et dont des vaisseaux étaient absents. Les cellules ressemblaient au parenchyme et, en coupe transversale, formaient des chaînes rayonnantes traversant plusieurs anneaux annuels successifs. Il se produisait généralement une certaine quantité de bois anormal durant plusieurs années et parfois les symptômes intenses se produisaient de nouveau sur l'emplacement d'une lésion plus ancienne.

ZUSAMMENFASSUNG

Anatomie und äußeres Erscheinungsbild der Buchenrinde wurden im Hinblick auf die unterschiedliche Anfälligkeit verschiedener Klone gegenüber der Buchenwollschildlaus untersucht. Äußerlich neigten die resistenteren Klone zu glatterer Rinde und wiesen weniger oder regelmäßig senkrecht verlaufende "Wachstumslinien" auf. Im Inneren unterschieden sich die resistenteren Klone von den anfälligen durch eine dünnere und/oder an verholzten Zellen reichere Parenchymschicht außerhalb der Hauptsteinzellschicht. Für die Ernährung der Läuse scheint diese Parenchymschicht von Bedeutung zu sein und es wird angenommen, daß die Ernährung erst dann ohne Schwierigkeiten erfolgen kann, wenn das Parenchym eine bestimmte Dicke erreicht hat. Ähnliche Beobachtungen wurden auch an unverklonten Bäumen gemacht.

Die Anatomie des Holzes wurde untersucht, um die anatomischen Ursachen für das "dimpling"-Symptom, Eindellungen am Stamm von lausbefallenen Buchen, zu klären. Unter den zentralen Bereichen der Vertiefungen zeigten ein oder zwei Jahresringe keine Gefäße und eine stark verringerte Zahl an Xylemzellen. Die Zellen in diesem Bereich ähneln Parenchymzellen und bilden im Querschnitt markstrahlähnliche Ketten über die Jahresringgrenzen hinweg. Bis zu einem gewissen Grade erfolgt anormale Holzbildung mehrere Jahre lang und in manchen Fällen sind über älteren Eindellungen starke frische Symptome zu beobachten.

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