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The Shape of Soft-rot Cavities – A Hypothesis

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Introduction

It has been known for a long time that a group of lower fungi now called soft-rot fungi, chiefly members of the Ascomycetes and Fungi Imperfecti, produce a decay pattern in the S2 layer of wood cell walls consisting of cavities which in surface view of the wall are diamond shaped with smooth sides and pointed corners or have parallel sides with pointed ends. These may occur singly or in chains and the longer axis of the cavity and of the chains lies in the direction of the preferred orientation of the cellulose microfibrils constituting the S2 layer. The cavities were first described by Schacht and the developments in the literature since then have been reviewed by Levy (1965) and Findlay (1970). The mode of attack on the walls by soft-rot fungi is now well established (Corbett, 1963; 1965; Corbett, Levy, 1963; Greaves, Levy, 1965; Levy, Stevens, 1966). A hypha lying in the lumen sends out laterally a fine hypha which bores through the S3 layer into the S2 layer where it forms a T-branch. The arms of the branch grow through the wall in each direction parallel to the microfibrils of the wall as broader hyphae which then form the cavities. It is the purpose of this paper to present a testable, semi-quantitative hypothesis to explain the shape of these cavities in terms of the known architecture of the S2 layer of the wood cell wall.

Hardwoods are known to be more susceptible to soft-rot attack than are softwoods, though softwoods do yield cavities (Savory, 1954), and this is commonly attributed to the lower lignin content of the walls of hardwoods. Non-wood fibres –even cotton hairs, which do not contain lignin–are also attacked by soft-rot organisms with cavity formation (Nilsson, 1974) though no cavities are produced with delignified wood or with rubbery wood (in which lignin synthesis is disturbed) (Findlay, 1970). The relation between lignin content and cavity formation is therefore complex. It is known, however, that the enzymes excreted to the substrate by,

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for instance, *Chaetomium globosum* show activity mainly with polysaccharides (Levi, Preston, 1965; Seifert, 1966) and that in wood subjected to soft-rot attack cellulose, xylan, araban and galactan are removed but lignin is only modified in some way (Levi, Preston, 1965). Nilsson has demonstrated more recently that a wide variety of soft-rot fungi produce xylanase and mannanase, and his finding of extensive cellulase activity has corrected an earlier impression that the cellulolytic activity of these organisms is low (Nilsson, 1974). He has specifically identified cellulase, xylanase and mannanase.

The angle subtended by the sloping sides of the cavities varies with tree species and perhaps between fungal types; according to Courtois (1963) this angle (2θ in Fig. 2) ranges from 47° (oak, pine larch) to 72° (beech). The development of cavities with a shape reminiscent of the crystalline state when neither fungus nor host exhibits any sign of external crystalline form, has attracted a great deal of attention. Two major hypotheses have been advanced in explanation of the cavities, each of which throws weight on the wall rather than on the fungus. In one of these, first proposed by Bailey and Vestal (1937) it is imagined that there are planes in the cellulose lattice which are preferentially hydrolised. This was given strong support by Frey-Wyssling (1938) and accepted both by Meier (1955) and by Wardrop and Jutte (1968) and Jutte and Wardrop (1970); the last two based their opinion on the oblique ends left on cellulose microfibrils after attack by enzymes, close to the values given by the molecular planes in the lattice identified by Frey-Wyssling. The range of values found by Courtois (loc. cit.) for 2θ speaks against this explanation, and it is in any case difficult to envisage smooth planes of attack across an assembly of (more or less) parallel microfibrils such as constitutes the S2 layer without a mutual register between microfibrils for which there is no evidence.

The other hypothesis postulates that the enzymes released by the fungus diffuse at different rates along and across the microfibrils and that these rates are so regulated that they produce a diamond-shaped hydrolysis cavity. This was first suggested by Roelofsen (1956) who proposed that enzymes can pass across the microfibrils only at points of contact between them; he placed the whole emphasis on cellulose in the wall and cellulase from the fungus. This hypothesis was supported by Levi and Preston (1965) who took into account the need for enzymes other than cellulase and who considered that the transfer of enzyme attack from one microfibril to the next must occur at a paracrystalline region (see below) of the recipient microfibril. It is this hypothesis which is to be taken up in this paper and given mathematical formulation.

The Structure of the S2 Layer

The formulation depends upon the structure of the S2 layer and this must therefore be recapitulated as far as is necessary in presenting the hypothesis. The constituent microfibrils lie more or less parallel to each other in a helix whose pitch varies with

cell length but is usually steep. They are disposed in lamellae and there is some variation in pitch from lamella to lamella. Individual microfibrils contain a central core which is crystalline, interrupted at irregular lengths by regions in which an admixture with polysaccharide chains other than cellulose induce a lower crystallinity or reduce the crystallinity to zero. These regions we shall call paracrystalline. The surface of a microfibril carries also polysaccharide chains other than cellulose which cannot be removed without destruction of the microfibril. These bring the microfibril up to a width of some 10 nm and a thickness of some 5 nm. Microfibrils are separated in the wall by hemicelluloses and by lignin though there is no evidence that they do not occasionally come into direct contact.

Theoretical Considerations

Complete understanding of the geometry of the development of a soft-rot hypha within the S2 layer involves answers to three main questions: (1) how does the invading hypha come to lie parallel to microfibril direction? (2) by what means is a single diamond-shaped cavity produced and (3) what induces the development of a chain of cavities? The present paper is intended to make a proposal about (2) only, but some attention will be paid to (1) and (3).

Consider a wall constituted uniformly of an array of straight, virtually endless microfibrils b nm wide spaced on an average d nm apart (Fig. 1). Suppose that each microfibril consists of a chain of crystallites of variable lengths $l_1, l_2, \ldots l_r \ldots l_n$, nm, with linking paracrystalline regions x nm long in the direction of the microfibril. We assume no fixed relation between the paracrystalline regions of one microfibril and the next. Suppose that lying within the array is a fungal hypha, initially in close contact with the array, of which one edge is shown at FF in Figure 1. Let us imagine in the first instance (though this requirement will later be removed) that enzymes are released at only one point P on the surface of the hypha (at an annular ring in the whole hypha) capable of destroying all the wall poly-

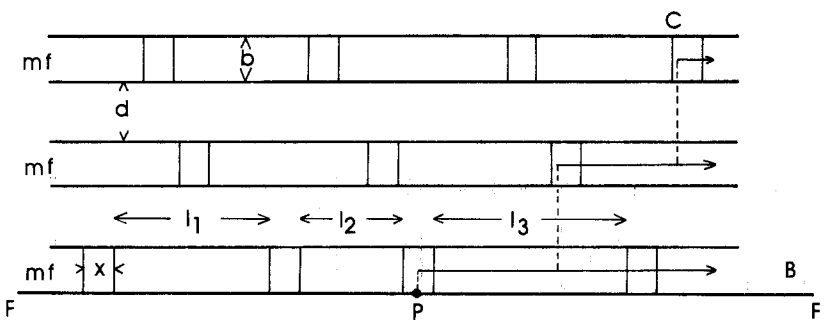

Fig. 1. Diagrammatic representation of the surface of a soft rot fungus hypha, FF, associated with an array of microfibrils, mf, in the S2 layer of a wood cell wall. l_1, l_2 etc. are the lengths of crystalline regions, separated by paracrystalline regions of length x. The single arrows show the paths of attack by enzymes emanating from point P

saccharides and degrading lignin. This enzyme complex is considered to be endowed with the following properties:

1) It can digest cellulose and hemicelluloses and can at least disorganise lignin.

2) It can attack polysaccharide chains at an end but cannot cut a cellulose chain in a crystallite anywhere along its length because either the structure of the enzyme or the close packing of the chains is prohibitive.

3) The enzyme can therefore initiate an attack on a microfibril only at a paracrystalline region. This is in harmony with the fact that when celluloses are attacked by fungi the crystallinity at first apparently increases.

The released enzyme then "finds" a paracrystalline region close to the hypha surface and from there digestion of the neighbouring crystallites occurs through the removal of glucose residues one by one along the chains. This initial attack on one microfibril can extend to the next microfibril in the array only when the enzyme passing along the first microfibril has reached a paracrystalline region in the second. The quickest "path" through the array at right angles to the microfibril will then be as shown by the broken lines in Figure 1, in comparison with the paths along the microfibrils shown by solid lines. We now proceed as follows.

Let t_1 be the time required to digest the whole of one paracrystalline region, t_2 the time required to digest unit length of a crystallite and t_3 the time required for the enzyme to traverse the distance d from one microfibril to the next. Then the shortest time required for the enzyme to pass along the microfibril from A to some arbitrary point B is given by

$$t = \tfrac{1}{2} t_1 + t_2 (l_1 + l_2 + \ldots + l_r + \ldots + l_n) + n t_1 = n(\bar{l} t_2 + t_1) ,$$

ignoring the $\frac{1}{2} t_1$ required for the initial digestion of the first paracrystalline region. The path involves n crystalline regions, average length $\bar{l}$ and an equivalent number of paracrystalline regions.

The time required for the enzyme complex to pass across the microfibrils to any arbitrary point C is similarly given by

$$t' = n' t_3 + \tfrac{1}{2} n' t_1 + \tfrac{1}{4} n' \bar{l} t_2$$

where n' is the number of microfibrils traversed. The factor $\frac{1}{4}$ in the third term arises because the quickest line of attack from one microfibril to the next lies in the direction of the nearest paracrystalline region of this next microfibril, along a length of the first microfibril which lies between 0 and $\frac{1}{2}l$ and is therefore on an average $\frac{1}{4}l$.

If the enzymes reach B and C simultaneously, then these two times must be the same. Hence

$$n' t_3 + (\tfrac{1}{2} n' - n) t_1 + (\tfrac{1}{4} n' - n) \bar{l} t_2 = 0 . \qquad (1)$$

The distance travelled in this common time is $n(\bar{l} + x)$ along the microfibrils and $n'(b + d)$ across the microfibrils. We can therefore set up a triangle as in Figure 2.

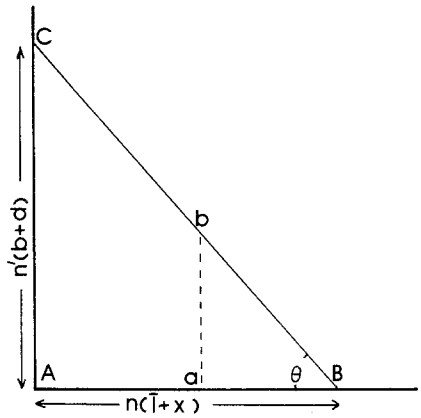

Fig. 2. For explanation, see text

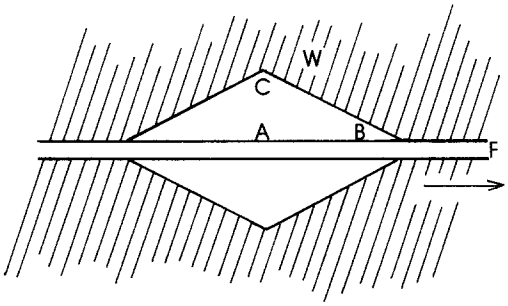

Fig. 3. Diagrammatic representation of a fungal hypha, F, and the associated cavity in surface view of the wall. W, unattacked wall; A, B, and C are as in Fig. 2

The line BC clearly represents the limit of the attack on the microfibril array at this time; for any enzyme complex reaching any arbitrary point *a* along the array and then proceeding across the microfibrils will reach point *b* on line BC at precisely the same time as the enzymes will reach B and C. The cavity is therefore limited by a mathematically straight line given by

$$y = n'(b + d) - x(n'(b + d))/(n(\bar{l} + x))$$

making an angle θ with the microfibril direction given by

$$\tan\theta = n'(b + d)/n(\bar{l} + x) \; . \qquad (2)$$

The total attack made by the whole hypha is therefore a diamond-shaped cavity as seen in surface view of the wall, as observed (Fig. 3).

Equation (1) sets a strict limit to the conditions under which a cavity can be acheived. If $n' \geqslant 4n$ (as set by conditions not represented in the equation) then one of the t's must be negative or all must be zero. The limiting condition $n' = 4n$

implies, from Eq. (2), that for any fixed value of the cell wall parameters the limiting value of θ is given by

$$\tan\theta = 4(b + d)/(\bar{l} + x) .$$

θ can be less than this but not greater. If, for instance, 2θ is to be required at 60° then

$$(\bar{l} + x) < 4\sqrt{3}\,(b + d)$$

must follow. If (b + d) is 20 nm, $(\bar{l} + x)$ must be less than 138 nm. Limiting values for θ are listed in Table 1 for a series of values of (b + d) and $(\bar{l} + x)$. Values bounded by the heavy line represent possible situations for $\theta = 47°$. Within this range, any value of θ may be achieved, ranging from 47° to the value given at any point in the Table, by suitable choices of t_1, t_2, and t_3. It may be worth noting that for a wall containing 40% cellulose the value of (b + d) in the plane of the wall is about 15 nm and the value of n'/n for $2\theta = 60°$ then ranges from 2.31 for $(\bar{l} + x) = 60$ to 3.85 for $(\bar{l} + x) = 100$ nm. Taking an average value of $n'/n = 3.0$, Eq. (1) gives

$$3\,t_3 + 0.5\,t_1 = 0.25\,\bar{l}t_2 .$$

$\bar{l}t_2$ is therefore greater than either t_1 or t_3. The varying activities of the enzyme complex must be such as to satisfy this equation for $2\theta = 60°$, and this gives a possible test of the theory.

Differences in θ between species may arise through a whole complex of factors. This may perhaps best be visualised if we define an overall rate of enzyme reaction as r_l units per second along the microfibrils and r_a across them. Then

$$n(\bar{l} + x) = n(\bar{l}t_2 + t_1)\,r_l$$

$$n'(b + d) = n'(t_3 + \tfrac{1}{2}t_1 + \tfrac{1}{4}t_2)\,r_a$$

whence

$$\tan\theta = \frac{n'(t_3 + \frac{1}{2}t_1 + \frac{1}{4}t_2)\,r_a}{n(\bar{l}t_2 + t_1)\,r_l}$$

Table 1. Limiting values of 2θ, rounded off to the nearest whole number, for different values of $(\bar{l} + x)$ and (b + d) nm

(b + d)	$(\bar{l} + x)$					
	60	70	80	90	100	1000
8	56	48	42	38	34	4
10	67	60	53	48	44	5
12	77	68	62	56	50	5
16	92	84	76	70	64	7
20	106	96	90	82	76	9
24	114	106	100	92	86	11

θ is therefore dependent on the nature both of the enzyme complex and of wall architecture.

The Three-dimensional Shape of the Cavity

Since only the S2 layer of the wall is eroded, the cavity as seen in sections cut parallel to the microfibrils will clearly be parallel sided with pointed ends (Fig. 4). The angle θ' may or may not be the same as θ. As viewed along the microfibrils, however, the angle subtended at the sides of the cavity should not be the same as either θ or θ' since it depends on the distribution of wall constituents at right angles to the microfibril direction and is therefore independent of $(\bar{l} + x)$. The sides could in some circumstances be rounded.

This follows the initial assumption that enzymes are secreted from only one narrow ring round the fungal hypha and that this ring does not move relative to the wall during cavity formation (Fig. 5 a). If the ring is, however, widened to an annulus then the cavity, even when viewed perpendicular to wall surface will be parallel sided with pointed ends. The shape of the cavity will then be as in Figure 5 b. If the fungal hypha is growing during cavity formation then there will be no effect on the cavity unless growth is so fast that the zone of release of the enzyme passes beyond the limits of the cavity already produced.

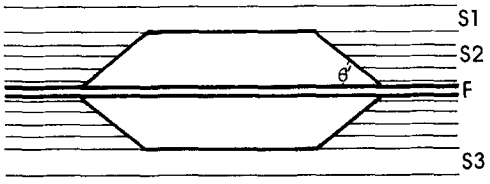

Fig. 4. Diagrammatic representation of a soft rot cavity viewed in a section of the wall parallel to the microfibrils

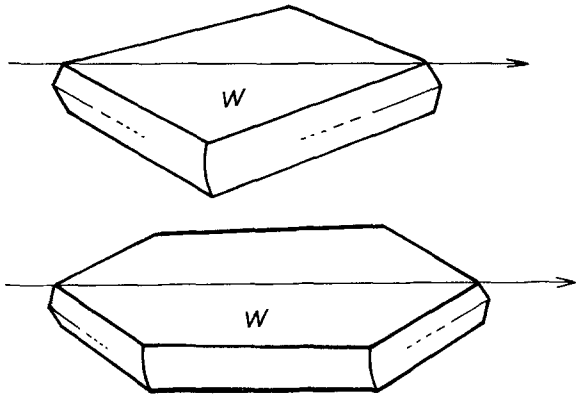

Fig. 5 a and b. The shape of a soft rot cavity in three dimensions. W, wall surface; the arrow shows the microfibril direction. **a** If the enzymes are released from a narrow ring round the hypha, **b** If the release is from a wide annulus

Discussion

The proposed hypothesis therefore predicts that a fungus will produce angular cavities in the wall—and thus should be classed as a soft-rot fungus—provided that two necessary prerequisites are met. The first of these is that the digesting hypha should come to lie within the cell wall. This is achieved through the bore-hole hypha of limited growth and the T-branching of its tip in the S2 layer. The second is that all possible paths of digestion illustrated in Figure 1 should be equally probable. This implies that the rate of production of enzyme molecules by the fungus, over an annulus of limited width behind the apex of the hypha, must be such that all paths are explored. The cavity is then a function of the nature of the fungus, the morphology of the wall and the statictics of the situation. To this extent, the hypothesis can be tested when it becomes possible to determine the rate of action of the enzyme complexes and to locate the site of secretion. Equally, accurate knowledge of the wall parameters (b + d) and ($\bar{l}$ + x) should allow at least a qualitative check.

These considerations began with a fungal hypha lying in the cell wall parallel to the microfibril direction and of themselves constitute only a partial explanation of cavity formation. It is not, however, conceptually difficult to see how this parallel alignment may come about, given that the bore-hole hypha branches in the S2 layer. If the branch primordia already secrete the wall-digesting enzymes then this will initiate cavity formation. Since the cavity has its greatest dimension in the direction of microfibril length, each branch will be constrained to orient itself in this direction. Alternatively, some other genetic message may determine the orientation of the two arms of the T-branch and cavity initiation may be achieved only when this is read and acted upon.

The production of chains of cavities rather than the continual extension of a single cavity suggests strongly that the release of enzymes necessary for cavity formation is periodic as suggested by Zainal (1978). Her contention that cavity formation is halted while a fine 'proboscis' hypha (Corbett 1963; Crossley, Levy 1977) grows through the wall to produce another 'digestive' hypha has been supported by an actual filming of the process by Leightley and Eaton (1977) and seems well conceived. In a sense, this means a recapitulation of the early invasion by the bore-hole hypha.

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