Some effects of fungal growth on the roots of *Rauwolfia oxyphylla* Stapf.

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DURING an investigation of the dried roots of *Rauwolfia oxyphylla* Stapf., a species indigenous to the swampy forests of Uganda, it was noticed that, with some samples, the xylem fibres isolated by Schulze maceration possessed abnormal characters, which appeared to be associated with certain micro-fungi. We have examined this association.

The external appearance of the roots was normal but the root-bark separated easily from the wood, often crumbling into flaky pieces. The internal surface of the bark was flaky and possessed white and black patches which corresponded with similar areas on the underlying wood surface. In ultraviolet light the inner surface of the bark, apart from white and black patches, appeared a deep orange instead of the normal dark brown. Smoothed transverse surfaces of roots frequently showed thin black lines running irregularly across them. From these roots, three cellulose-destroying micro-fungi. Melanospora zamiae Corda, Chaetomium funicolum Cooke and C. globosum Kunze were isolated by Dr. L. Jacobs and identified by the Commonwealth Mycological Institute. These species are normally found on plants rotting in damp conditions. They belong to that group of wood-attacking micro-fungi which cause "soft-rot" involving the production of pointed cavities within the middle layer of the secondary wall. Timber attack by these fungi has been known for many years and Schacht, in 1850, reported the characteristic pointed cavities. A detailed study has been made by Bailey & Vestal (1937); the term "soft-rot" was introduced by Savory (1954). Three other groups of fungi which attack wood are recognised by Mr. J. F. Levy (personal communication), but these are outside the scope of this report.

To study the effects of the infestation of *Rauwolfia oxyphylla* root by the individual fungi, the following procedure was adopted. Discs of normal *R. oxyphylla* root, about 3 mm in thickness, were sterilised in sealed polythene packets by irradiation (1·1 megarads over 2 hr). After being moistened with sterile water, the discs were aseptically transferred to sterilised petri dishes containing a layer of water. The roots were supported above the level of the water and their surfaces inoculated with the appropriate fungus which had been cultivated in potato-dextrin-agar or malt-agar media. The strains used were those isolated from the roots. The dishes were incubated at 26°, the humidity within the incubator being maintained at about 80% to prevent the specimens drying out. Samples of wood were examined at 7-day intervals for abnormal wood fibres.

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FUNGAL GROWTH ON THE ROOTS OF R. OXYPHYLLA

The results are recorded in Table 1. It is evident that the three fungi can each attack the wood fibres of R. oxyphylla. Cavities in the fibre walls, identical with those observed in the fibre walls of the original root samples, were produced by each of the individual fungi in the experimental material.

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PRODUCTION OF ARNORMAL FIRES IN Raywolfia aryphylla ROOT-WOOD

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| Incubation | Abnormal fibres produced after fungal growth | | | | | | | |
|---------------------|--|----------------------------------|----------------------------------|---|--|--|--|--|
| (days) | Melanospora zamiae | Chaetomium funicolum | Chaetomium globosum | Control (No inoculation with fungi) | | | | |
| 7 14 21 28 | None None About 5% About 10% | None None About 10% 75% | None None About 50% 75% | None None None None | | | | |

It is possible to divide the digestion of the fibre wall into three stages (Fig. 1) as follows (Jefferies, 1965). An *early stage* in which numerous, narrow, elongated cavities with sharply pointed ends appear within the middle layer of the secondary cell wall. These are scattered along the length of the fibre, are relatively short, and do not give a spiral effect. They are all parallel to one another and in polarised light appear as dark lines in a "bright" fibre. A *middle stage* in which the cavities elongate so that some spiral effect is observed; some cavities may widen which makes the pointed ends even more prominent. The cavities remain parallel and in polarised light their dark spirals are very marked. A *late stage* in which the number of cavities is greatly increased so that many of them join to form wide areas of decomposed cell wall. The lines on the fibres are still approximately parallel and they are so frequent that it is difficult to distinguish normal cell wall areas from decomposed areas.



FIG. 1. Portions of wood fibres of *Rauwolfia oxyphylla* Stapf. All \times 200. 1, normal fibres; 2, 3, 4, fibres after four weeks incubation with *Chaetomium globosum* showing respectively, early, middle and late stages of fibre wall digestion. C, cavity; P, pit.

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In polarised light these areas can be distinguished easily by the brightness of the unattacked cell walls. Eventually the unattacked residues of the cell walls are reduced to narrow spiral strips, still running parallel to one another; because the hardly visible, outer and inner layers of the cell walls remain to a large extent unattacked, they hold these few spirals of unattacked fibre wall together. Finally, even these areas are digested and the fibres in this condition do not survive Schulze maceration.

In transverse section the thin structure of the inner layer of the cell wall can be seen clearly either as an intact ring or broken (by the microtome) and lying within the lumen of the fibre, but in any event quite detached from the remainder of the cell wall. It does not appear bright in polarised light. The early stage of attack is difficult to see but can be detected by careful examination of suitable transverse sections. With the fungi studied, xylem parenchyma and xylem medullary ray cells do not normally show any effect of attack and vessels only rarely possess fine spiral cavities. Microchemical stains are available for the selective staining of fungal hyphae, making detection easier in cases of slight attack.

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