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SHORT COMMUNICATION

THE ROLE OF EXTRACTIVES IN THE FORMATION OF ECTOTROPHIC MYCORRHIZAE

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DURING electron microscope studies of the fine structure of mycorrhizae of *Pinus radiata* Foster and Marks[1,2] noted that, after fixing, the cells of the "tannin" layer[3] in the outermost layer of the cortex were filled with very electron-dense materials. The accumulation of these substances was considerably less in the inner cortical cells and practically absent in the fungal mantle. They also observed that the mycorrhizae fungus showed symptoms of being affected by toxins as it passed through the "tannin" layer, but not so once it had passed into the inner cortical cells where it formed the Hartig net. In *Pseudotsuga menziesii* they found evidence of severe toxicity to the fungus, serious enough to stop the passage of carbohydrate from the Hartig net to the mantle, or, in other cases, to stop intercellular penetration by the mantle fungus. Furthermore, the tannin layer was discontinuous in healthy *P. menziesii*, whereas in chlorotic seedlings it formed a complete ring around the periphery of the cortex.

In Australasia, *P. radiata* can be established with ease in afforestation programmes, whereas considerable difficulty is encountered with *P. menziesii* due to mycorrhizal problems.[4] Since the evidence from the fine structure studies suggests that this difference is due to interference with mycorrhiza formation by the components in the tannin layer, a comparison was made of these compounds in samples of mycorrhizae from the two species.

Mycorrhizae from three-year old *P. menziesii* and *P. radiata* trees were collected from three nurseries from different climatic regions and with different soil types so that the results would be ecologically more valid. The mycorrhizae were stripped off the roots along with the adhering soil and collected using a wet sieving method,[5] eventually being obtained with less than 5 per cent impurities. Within 24 hr, the mycorrhizae were freeze-dried, and stored at under 0° in the dark. They were then extracted repeatedly with acetone at 15–20°. Only small amounts (100–200 mg) of extractives could be obtained, and consequently analysis was carried out by chromatographic and spectrophotometric methods.

[1] R. C. FOSTER and G. C. MARKS, *Australian J. Biol. Sci.* **19**, 1027 (1966).
[2] R. C. FOSTER and G. C. MARKS, *Australian J. Biol. Sci.* (in press).
[3] D. T. MACDOUGAL and J. DUFRENOY, *Plant Physiol.* **19**, 440 (1944).
[4] J. W. GILMOUR, *New Zealand J. Forestry* **7**, 94 (1958).
[5] G. C. MARKS, NELL DITCHBURNE and R. C. FOSTER, *Australian Forestry* (in press).

The total amount of mycorrhizae acetone-soluble extractives in *P. menziesii* and *P. radiata* was between 2·6 and 6·0 per cent. The heptane soluble ("resin") portion made up 54–67 per cent of the extractives of *P. menziesii* and 87 per cent and 45 per cent in vigorous and slow-growing *P. radiata* respectively. Only five components were evident in the non-resinous portion of *P. radiata* root extractives; catechin, two stilbene-like components, a coumarin-like component and a leucoanthocyanin. The non-resinous portion of the extractives of *P. menziesii* contained fifteen components, including catechin, flavonoids and appreciable amounts (about 6 per cent of the extractives) of an unstable compound with properties consistent with that of a pentaene.[6] The composition of the non-resinous root extractives in each species was the same in the mycorrhizae collected from good and poor sites and with different fungal types and appears to be characteristic. However, the evidence so far obtained suggests that the mycorrhizae from poor sites contain more non-resinous extractives than those from good sites.

The extractives could have come from either the host or the fungus. The fine structure studies on material prepared with fixatives, showed considerable concentrations of unsaturated or reactive substances in the epidermal layer of the root tissues but very little, if any, in the fungus, indicating that the host is probably the origin of the extractives. The presence of the stilbene-like compound is significant as some of these compounds have fungitoxic activity. Also, compounds that can be considered as dihydrostilbenes have been isolated from orchid roots after fungal infection.[7] The situation regarding the "pentaene" is different. Such substances have been produced by fungi[6] and are known to have antibiotic and antifungal properties. On the other hand, our fine structure studies indicate that the pentaene is present in the host, and highly unsaturated compounds of different types have been isolated from roots of various plants although not from the Coniferae.[8]

The *P. menziesii* mycorrhizae which contains a wider range of polyphenols and other non-resinous extractives than radiata pine, would seem to provide a more discriminatory biochemical screen to fungal penetration. This may make it harder for the former species to find suitable fungal associates in a new environment.

The work will be reported in detail later.

[6] D. S. BHATE in *Chemistry of Natural and Synthetic Colouring Matters* (edited by T. S. GORE, B. S. JOSHI S. V. SUNTHANKAR and B. D. TILAK) p. 341, Academic Press, New York (1962); A. C. COPE and H. E. JOHNSON, *J. Am. Chem. Soc.* **80**, 1504 (1958); G. B. WHITFIELD *et al. J. Am. Chem. Soc.* **77**, 4799 (1955).

[7] E. HARDEGGER, M. SCHELLENBAUM and H. CORRODI, *Helv. Chim. Acta* **46**, 1171 (1963); J. URECH, B. FECHTIG, J. NÜESCH and E. VISCHER, *Helv. Chim. Acta* **46**, 2758 (1963).

[8] N. A. SORENSEN in *Chemical Plant Taxonomy* (edited by T. SWAIN) p. 219, Academic Press, New York (1963).