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Ultrastructural and biochemical changes at the preinfection stage of mycorrhizal formation by two isolates of *Pisolithus tinctorius*

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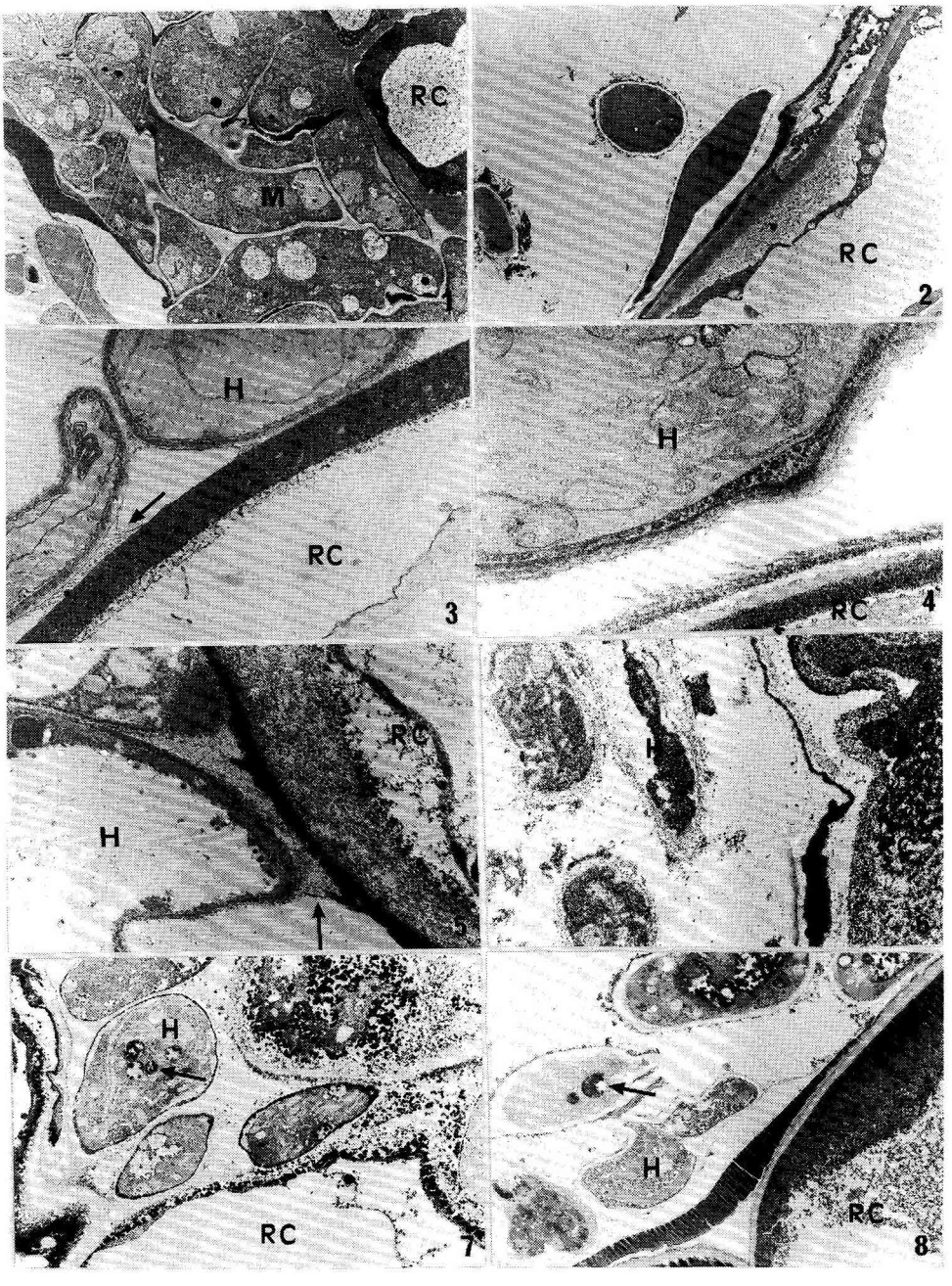
Introduction

In a previous paper, Malajczuk *et al.* (1989) demonstrated major infection differences existing between isolates of *Pisolithus tinctorius* on roots of *Eucalyptus urophylla*. A strain originally isolated from under *Eucalyptus* in Australia (445) appeared to be much more aggressive toward this host plant than one isolated from under *Pinus* in North America (270), despite the fact that ultimately the mycorrhizal infection occurs in both cases. To date, there have been no hypotheses to explain ectomycorrhizal specificity and aggressiveness, although the action of anti-fungal compounds originating from the plant has been suggested (Duchesne *et al.*, 1987). Information from plant pathology literature has implicated chemical messengers as being responsible for specificity and aggressivity (Halverson and Stacey, 1986). Recently, there have been a number of publications examining early stages of mycorrhizal infection especially using *Eucalyptus* as the plant model (Malajczuk *et al.*, 1984; Massicotte *et al.*, 1987; Horan *et al.*, 1988). In all these studies, however, the very early stages of plant–fungus contact, *i.e.*, occurring before the ectomycorrhizal infection *sensus stricto*, have been overlooked. It would seems reasonable to assume that specificity and aggressivity of the infection process is determined and could therefore be characterized, during the very early events of plant–fungus interaction, as soon as recognition between both symbionts had occurred.

Materials and Methods

Plant materials and mycorrhizal synthesis were according to Malajczuk *et al.* (1989). Fine lateral roots were sampled after 2 and 4 d of incubation and processed according to Lei (1988) for electron microscopy observations, ultra-



Figs. 1–8. RC: root cortical cell; H: hyphae; M: fungal mantle.

structural localization of polysaccharides and proteins, and measurement of acid phosphatase activities.

Results

Four days after inoculation with strain 445, the fungal sheath was well established and compact (Fig. 1, x 3200). The typical one-layer Hartig net was well developed. With strain 270, most of the hyphae were still some distance from the root surface. Where the hyphae are in contact with the root, a thickening of the host plant cell wall can be observed, resulting from deposition of dense granular or fibrillar materials on the internal wall face (Fig. 2, x 3100).

Two days after inoculation with strain 445, hyphae were in contact with cortical cells and polysaccharidic fibrillar material, reacting in the PATAg test, can be observed between both organisms (Fig. 3, x 25 000). These fibrils reacted positively in the Swift test indicating the presence of cystine-rich proteins (Fig. 5, x 13 000). With strain 270, 4 days after inoculation, root cell walls as well as fungal cell walls were highly reactive in the PATAg test, but no fibrillar materials could be detected between root and fungal cells (Fig. 4, x 20 000). The Swift test was equally non-reactive in this zone (Fig. 6, x 10 000).

Four days after inoculation with strain 445, acid phosphatase activity was in evidence at the surface of the fungal plasmalemma as well as in the vacuoles. The plasmalemmal activity was detected when the hyphae were close to the roots, while it was nearly absent in hyphae distant from the root (Fig. 7, x 4000). In the host–plant cells, acid phosphatase activities were localized along the plasmalemma. With strain 270, acid phosphatase activity was detected only in fungal vacuoles (Fig. 8, x 4600).

Discussion and Conclusion

The ultrastructural comparison of the early events of infection between *E. urophylla* and both strains of *P. tinctorius* shows important differences at the interface. Indeed, it seems that the plant reacts to the presence of *P. tinctorius* isolated from under pine, as if it were in contact with a pathogenic strain. Field results shows that this strain has a poor ability to resist competition with indigenous fungi associated with *Eucalyptus* in plantations in the Congo (Garbaye *et al.*, 1988). Understanding recognition is therefore quite important for the controlled utilization of mycorrhizal symbionts in plantation management.

Presently, we do not have any knowledge regarding the nature of the signal from each organism, which initiates this succession of early events of mycorrhizal infection and which determines this selective aggressiveness. It can be suggested that cell surface glycoproteins are playing an important role during recognition but no evidence has yet been given.

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