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## ERICOID MYCORRHIZAL FUNGI OF *GAULTHERIA SHALLON*

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Salal (*Gaultheria shallon* Pursh) is one of the most abundant forest undergrowth species on the west coast area of North America. Large areas of productive forest land are invaded by these vigorously growing plants, especially following clearcutting and slashburning (Bunnell, 1990; Weetman et al., 1989), and in the presence of salal, the growth of conifer plantations is dramatically reduced (Weetman et al., 1989). Salal, like some other ericaceous plants, such as *Calluna vulgaris* (L.) Hull (Handley, 1963) and *Kalmia angustifolia* L. (Mallik, 1991), is of ecological importance (Smith, 1991), and, therefore, it is of interest to understand the basis of the success of salal in such sites.

Ericoid mycorrhiza formation contributes to the success of ericaceous plants in stressed soils (Read and Bajwa, 1985; Read, 1984). Salal forms ericoid mycorrhizae (Largent et al., 1980); however, it is not known which fungi are involved. The purpose of the present study was to identify the mycorrhizal fungi of salal from a cutblock on northeast Vancouver Island by isolation of fungi from roots and aseptic synthesis of mycorrhizae of salal with the isolates.

Salal roots were collected September 20–24, 1990, from 3-year-old western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) reforestation sites at Port McNeill on Vancouver Island, Canada. Sixty root samples from different locations were sorted to remove rhizomes and coarse debris, soaked in tap water in a flask for 10 min to loosen adhering fine debris, shaken vigorously in the flask, and washed in running tap water for 1 h. Roots were surface sterilized in a laminar flow hood by immersing them in 30% hydrogen peroxide in an autoclaved beaker for 30 sec. Sterilized roots were then washed repeatedly in sterile distilled water. Approximately 0.5 cm lengths were excised from the disinfected roots, and 10 pieces per Petri plate were placed onto 1/3 strength

potato dextrose agar (PDA). Plates were incubated at 25 C in the dark. Pure colonies were transferred onto modified Melin Norkrans agar (MMN) and incubated at 25 C for subsequent inoculation and identification (Pearson and Read, 1973).

Seeds of salal purchased from Reid Collins Nurseries in Aldergrove, British Columbia were soaked in tap water in a refrigerator until the seeds sank to the bottom of the flask (about 1 wk). The seeds were then rinsed repeatedly in tap water, drained and transferred in the flow hood into an autoclaved beaker half-filled with 30% hydrogen peroxide and sterilized for 30 sec. After sterilization, the seeds were rinsed four times in sterilized, distilled water. Finally, the seeds were drained and plated onto water agar. The Petri plates were kept in a growth chamber at 25 C and a light regime of 18 h light at 310  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  illumination and 6 h dark. After 2 wk the seeds started to germinate.

All fungal isolates recovered from plates were tested to form mycorrhizae with salal in pure culture. The synthesis was conducted in an 11  $\times$  2.5 cm screw-capped vial. Two cm of the vial was filled with 1.5% water agar and autoclaved for 30 min. When the agar solidified, 10 ml of sterilized soil (autoclaved for 60 min twice with an interval of 24 h) was placed in the vial. Once the soil had moistened by absorbing water from the underlying agar, one germinant was aseptically planted in each vial and kept in the growth chamber at 25 C with a light regime of 18 h light at 310  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  illumination, 6 h dark. When the first post-cotyledonary leaves emerged, seedlings were aseptically inoculated with one rectangular block each cut from edges of colonies of the isolates from salal. The closed, inoculated vials were returned to the growth chamber.

After 1 month, seedlings that had on the average eight leaves and a shoot that was 6 cm tall were harvested. The root system was washed in running tap water, dried with paper towel, and

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immersed in FDA Blue No. 1 staining solution (Chapman, 1992). The stained roots were mounted on a slide and examined under a compound microscope.

Eighty-three isolates of fungi were obtained from 60 root samples of field-grown salal and 24 of them produced typical ericoid mycorrhizae. These mycorrhizae were characterized by a web of hyaline hyphae on the surfaces of the hair roots and crowded hyphal complexes inside the outer layer of cortical cells. Morphological differences between mycorrhizae formed by the different isolates were not evident under the light microscope.

All 24 fungal isolates which formed ericoid mycorrhizae with salal grew slowly during the culture on MMN and PDA media. Among them, 18 isolates formed grey-olive colonies on PDA, dirty-white on malt extract agar, and dark olive on MMN. They formed conidia and conidiophores on all media tested and were identified as *Oidiodendron griseum* Robak as described by Barron (1962). Cultures have been deposited at the University of Alberta Microfungus Collection and Herbarium. Three other isolates exuded yellow-brown pigment into malt extract agar medium. The colony of another was reddish-brown and released a reddish-brown pigment into the media. The other two isolates were white. Since they did not sporulate, none of these six isolates could be identified.

*Oidiodendron griseum*, as a saprophyte, has been obtained from forest soil, paper samples, toilet tissue, wood pulp (Barron, 1962), tree leaves, humus, and rhizosphere soil (Dalpe, 1986; Douglas et al., 1989; Stoyke and Currah, 1991). As an ericoid mycorrhizal endophyte, it was isolated from *Vaccinium* species (Couture et al., 1983). This is the first report of *O. griseum* as an ericoid mycorrhizal fungus of *Gaultheria*.

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Key Words: Ericaceae, isolation, *Oidiodendron griseum*, salal

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