ENDOGONE FLAMMICORONA AS A MYCORRHIZAL SYMBIONT OF DOUGLAS FIR IN NEW ZEALAND

MYRA CHU-CHOU and LYNETTE J. GRACE

Forest Research Institute, New Zealand Forest Service, Rotorua

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ABSTRACT

Sporocarps of **Endogone flammicorona** Trappe & Gerdemann were found to be associated with roots of Douglas fir (**Pseudotsuga menziesii** (Mirb.)Franco) from its seedling stage to trees over 70 years of age. Seedlings of Douglas fir inoculated with pieces of sporocarps of **E. flammicorona** formed simple, unbranched ectomycorrhizas on short roots. The mycorrhizal seedlings were significantly taller and heavier than the control seedlings. This is the first report of the occurrence of **E. flammicorona** in New Zealand and of its association as an ectomycorrhizal fungus of Douglas fir in this country.

INTRODUCTION

Members of the Endogonaceae are well known for their role as endomycorrhizal symbionts of trees, shrubs, and herbaceous plants (Mosse, 1973). However, only recently has *Endogone lactiflua* Berk. & Broome been reported as a symbiotic fungus which forms ectomycorrhizal associations with Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and *Pinus strobus* L. (Fassi, 1965; Fassi and Palenzona, 1969; Fassi *et al.*, 1969). In New Zealand, members of Endogonaceae forming endomycorrihzas on native plants and agricultural crops have been extensively studied (Baylis, 1967; 1969; 1971; Powell, 1977; Cooper, 1976; Hall, 1977; Johnson, 1977; Morrison and English, 1967), but little attention has been given to the possible role of species of *Endogone* as ectomycorrhizal symbionts of exotic conifers which are of economic importance.

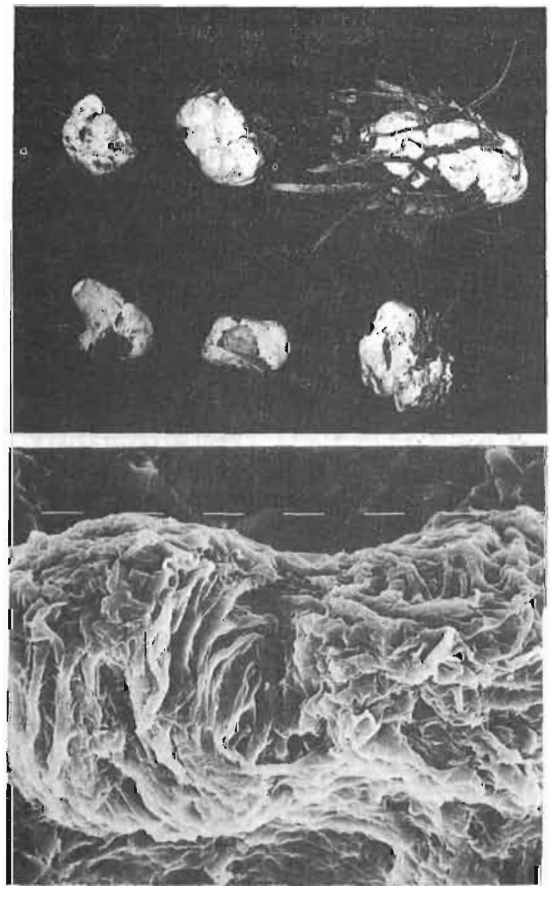
During a survey of mycorrhizal associates of Douglas fir seedlings in the Forest Research Institute nursery, Rotorua, sporocarps of *Endogone flammicorona* Trappe & Gerdemann (a segregate from *E. lactiflua, see* Trappe and Gerdemann, 1972) were observed associated with 2-year-old seedlings. A more extensive survey of the occurrence of this fungus was carried out in Douglas fir stands aged from 3 to over 70 years in Kaingaroa and Whakarewarewa State Forests, and its ability to form mycorrhizas with Douglas fir seedlings was tested. Sporocarps of *E. flammicorona* were collected by raking and searching through the leaf litter and upper layer of soil. The sporocarps were usually found under the litter layer next to the soil surface, very close to the fibrous and mycorrhizal roots of Douglas fir. Sporocarps were found only in Douglas fir stands over 5 years of age.

MORPHOLOGY OF SPOROCARPS

Sporocarps are at first white and more or less spherical, but later turn yellowish, and become lobed and corrugated; finally the entire sporocarp changes to a cinnamon colour and becomes irregular in shape (Fig. 1). The sporocarps often grow around small sticks of wood or decomposing needles which thus become embedded in them. The gleba of the sporocarp is creamy white in young specimens, becoming apricot yellow or orange and finally cinnamon-coloured with increasing maturity. The size of the sporocarps varies from 0.5 to 2 cm in diameter. In mature specimens, the zygospores in the sporocarps appear as orange-coloured granules. Microscopically the mature zygospores are seen to be characteristically wrapped in a spirally arranged mycelial mantle (Fig. 2) (Trappe and Gerdemann, 1972).

SYNTHESIS OF MYCORRHIZAS

Fungal inoculum — Fresh sporocarps were first surface-sterilised with 0.7% calcium hypochlorite for 5 minutes and then cut into small pieces (approx. 1 × 2 mm). From this material 40 0.5-g portions were weighed. Twenty portions were heated to 100°C



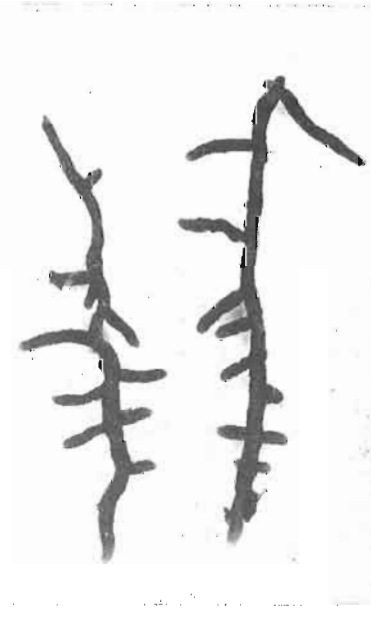


FIG. 1 (top left) — Sporocarps of **Endogone flammicorona**.

FIG. 2 (left) — Mature zygospores showing spirally arranged hyphal mantle. × 400.

FIG. 3 (above)—Mycorrhizas formed by **Endogone flammicorona** on Douglas fir seedling. × 5.

in an oven for 1 hour in order to kill the fungus, and the other 20 portions were not treated.

Douglas fir seedlings — Douglas fir seedlings were raised in the same way as in a previous study with *Pinus radiata* D.Don (Chu-Chou, 1979). The seedlings were planted in an autoclaved soil : peat : pumice (3 : 3 : 1) mixture in test tubes (40 × 200 mm). Seedlings were grown under fluorescent light (40 W/m^2, 12-hr photoperiod) at room temperature (19-20°C).

Inoculation method and treatments — At the age of 6 weeks each seedling was inoculated with 0.5 g fungal inoculum inserted near the root system. The treatments were (1) fresh sporocarp pieces, (2) heat-treated sporocarp pieces, and (3) non-inoculated control. Twenty seedlings were used for each treatment.

Results — The seedlings were removed from the test-tubes 3½ months after inoculation, and the root systems washed free of the substrate; heights, dry weights, and the mycorrhizal formation were assessed. The inoculated seedlings were significantly ($P < 0.01$) taller and heavier than the controls (both uninoculated and inoculated with heat-treated sporocarps) (Table 1). Between the controls there was also a significant ($P < 0.05$) height and weight difference — probably owing to the nutrients contained in the heat-treated sporocarps. Mycorrhizas were formed only on the seedlings inoculated with fresh sporocarps. The colour and morphology of the mycorrhizas was not very distinct from ordinary, uninfected short roots except for the absence of root hairs (Fig. 3). An examination of transverse sections of the synthesised mycorrhizas showed that there was no obvious fungal mantle, but the Hartig-net was well-developed as described by Fassi and Palenzona (1969).

TABLE 1—The effect of inoculation with **Endogone flammicorona** sporocarps on height and dry weight of Douglas fir seedlings (means of 20 seedlings)

Treatment	Height (mm)	Dry weight (g)
1. Fresh sporocarps	123	0.54
2. Heat-treated sporocarps	61	0.36
3. Uninoculated	49	0.26

NOTE: The differences between treatments 1 and 2 are highly significantly different ($P < 0.01$) and those between treatments 2 and 3 are significantly different ($P < 0.05$).

DISCUSSION

The presence of sporocarps of *E. flammicorona* in stands of different ages, and its ability to form ectomycorrhizas as shown by the synthesis test, indicate that this fungus may be an important mycorrhizal symbiont of Douglas fir in this country. Most of the members of Endogonaceae are recognised as extremely difficult to culture on artificial media (Barrett, 1961; Gerdemann, 1970) and to date our attempts to culture *E.*

flammicorona have not been successful. Therefore, it is impossible to use an isolation method to determine the proportion of Douglas fir mycorrhizas which are formed by *E. flammicorona* under natural conditions.

The difficulties in distinguishing mycorrhizas formed by *E. flammicorona* from uninfected short roots in the field mean that the relative importance of this fungus as an ectomycorrhizal symbiont of Douglas fir in New Zealand might be easily underestimated. The comparative beneficial effects of *E. flammicorona* and other mycorrhizal symbionts on the growth of Douglas fir seedlings have not been studied but this is a topic deserving attention.

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