

Short Communication

Mycorrhizae of *Monotropastrum globosum* growing in a *Fagus crenata* forestMaria Catarina Megumi Kasuya¹⁾, Kazuhiko Masaka²⁾ and Tsuneo Igarashi²⁾¹⁾ Dep. de Microbiologia, Univ. Federal de Viçosa, Viçosa, MG, 36570-000, Brazil²⁾ Dep. of Forest Science, Fac. of Agriculture, Hokkaido Univ., Kita-ku, Sapporo 060, Japan

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The achlorophyllous *Monotropastrum globosum* was found growing in a *Fagus crenata* forest. Samples of *M. globosum* and their interpenetrating root systems of *F. crenata* were collected to investigate the mycorrhizal association. *Monotropastrum globosum* mycorrhizae showed thick sheaths, invasion of the epidermal cells by fungal pegs, and Hartig nets, which reached only the first layer of cortical cells. The *F. crenata* mycorrhizae also showed thick sheaths, but Hartig nets penetrated deep into the cortex and intracellular hypha were seen in the outer cortical cells. The similarities observed in the mantle inner plan view and emanating hypha suggest that both mycorrhizae are formed by the same fungus.

Key Words—ectendomycorrhizae; ectomycorrhizae; *Fagus crenata*; *Monotropastrum globosum*; monotropoid mycorrhizae.

The waxy white appearance of *Monotropa* and *Monotropastrum*, a group of Pyrolaceae, subfamily Monotropoideae, indicates their achlorophyllous nature and has attracted the attention of mycorrhizal researchers (Björkman, 1960). Their inability to fix CO₂ photosynthetically means that they are dependent upon their mycorrhizal fungi for carbon compounds as well as other nutrients (Björkman, 1960; Robertson and Robertson, 1982; Peterson and Farquhar, 1994). These plants can be found under dense, moist forests with a surface accumulation of leaf litter, often in situations which are too shady for autotrophic growth (Leake, 1994). Such associations can also be found with *Armillaria* spp. and some achlorophyllous orchids, e.g., *Gastrodia elata* Blume (Harley, 1969; Cha et al., 1993; Cha, 1995) and *Galeola septentrionalis* Reichb. fil. (Terashita, 1985; Terashita and Chuman, 1987; Cha, 1995). Plants in such associations have been termed "epiparasites"; parasites on fungi which are themselves parasitic or mycorrhizic upon other hosts (Björkman, 1960; Harley, 1972).

The distribution of *Monotropastrum* is limited to the Himalayas and East Asia, while *Monotropa* sp. is widely distributed in the northern temperate zone (Ohwi and Kitagawa, 1983). In Hokkaido, northern Japan, *Monotropastrum globosum* H. Andr. ex Hara is observed in broad-leaved, coniferous or mixed forest. The flowering scape, which reached 10 to 20 cm in height (Fig. 1A), begins to appear in early summer (June) and matures (Fig. 1B) during July to August (Ohwi and Kitagawa, 1983). Tsukaya (1988) reported the appearance of a fruitbody of *Boletus calopus* Fr. beside *Monotropastrum humile* (D.

Don.) Hara and suggested that the bolete could be associated with *M. humile*.

This study was conducted to investigate the mycorrhizal structures of both *M. globosum* and the associated tree, *Fagus crenata* Blume, at the microscopic level.

Materials and Methods

Samples of *M. globosum* were collected under *F. crenata* forest in Utsai, Kuromatsunai-cho, Hokkaido, in early summer (July 1994). Blocks of soil were sampled including both root systems of *M. globosum* and *F. crenata*. The roots were taken to the laboratory and washed in running tap water, and the young root tips were washed several times with distilled water. One part was observed under a dissecting microscope and photographed. The other part was fixed in 2% glutaraldehyde in phosphate buffer (pH 6.8) and stored at 4°C. For microscopic observation, the protocol described by Agerer (1994) was used for cryotomy, and sections were stained with 0.5% Toluidine blue O in water, and observed under the light microscope and photographed. Terminology of structural mantle types used in this paper is based on Ingleby et al. (1990).

Results

Field and dissecting microscope observation revealed that *M. globosum* was connected to *F. crenata* by the hypha and/or rhizomorph, forming mycorrhizae in both plants. The root system of *F. crenata* interpenetrated

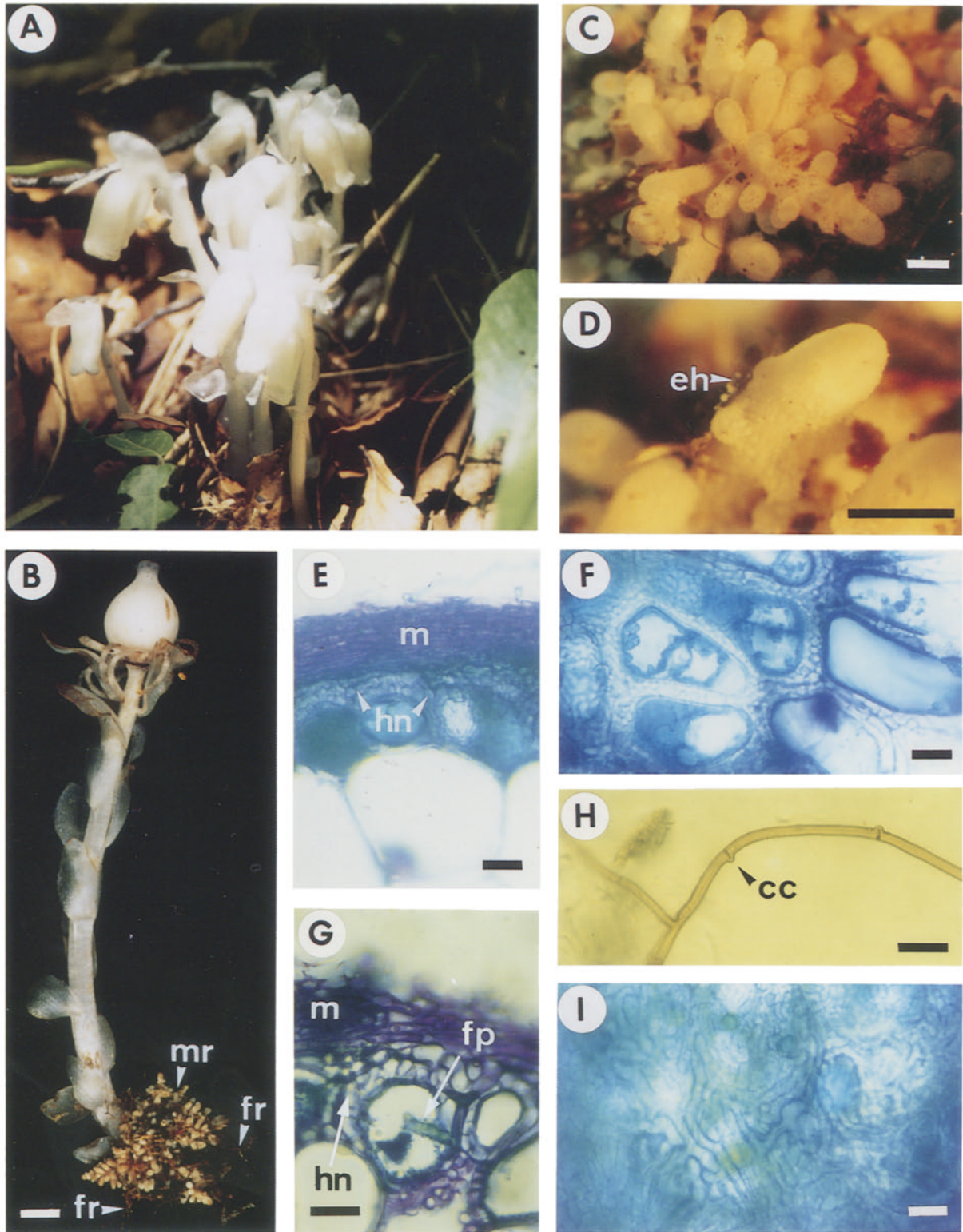


Fig. 1. (A) Flowering scape of *Monotropastrum globosum*; (B) Mature *M. globosum* and its root system (mr), showing the interpenetrating root system of *Fagus crenata* (fr). (C–I) Mycorrhiza of *M. globosum*: (C) The pyramidal shape; (D) Emanating hypha (eh) and irregular surface; (E) Transect showing the thick sheath (m) and reduced Hartig net (hn); (F) Transect showing the Hartig net with many hyphae crossing the intercellular space; (G) The fungal peg (fp) in the epidermal cell; (H) Emanating hypha with clamp connection (cc); (I) The inner plan view of the mantle showing the net synchyma structure. Scales: B = 1 cm; C, D = 1 mm; E, G, H = 50 μ m; F, I = 10 μ m.

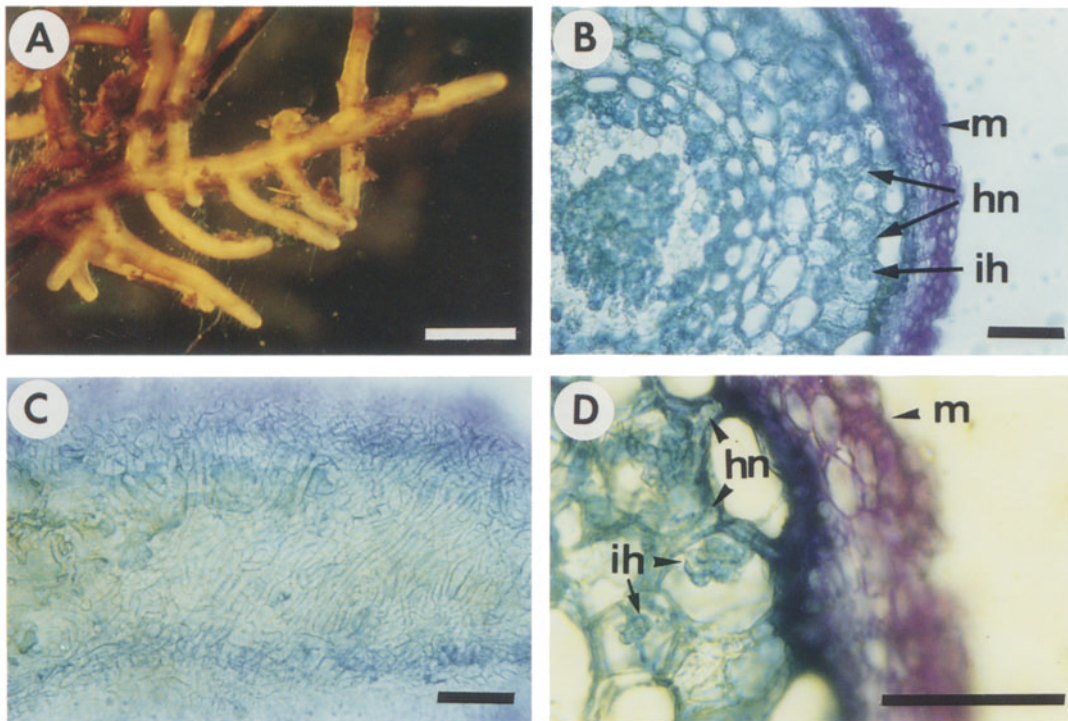


Fig. 2. Mycorrhiza of *Fagus crenata*: (A) Branched-monopodial mycorrhiza; (B) Transect showing thick mantle (m) and deep penetration of Hartig net (hn) into the cortex; (C) Inner plan view of the mantle showing the net synenchyma structure; and (D) Intracellular hyphae (ih) in the outer cortical cells. Scales: A = 1 mm; B–D = 30 μm .

with the root system of *M. globosum* (Fig. 1B).

Mycorrhiza of *Monotropastrum* The mycorrhiza of *M. globosum* is branched-pyramidal, pale yellow when young and yellow with age, with an irregular surface (Fig. 1C), and emanating hyphae (Fig. 1D). The transect of a mycorrhiza shows a dense mantle about 30 μm thick, with a reduced Hartig net involving only the epiderm and the first cell layer of the cortex (Fig. 1E), but this Hartig net is formed by many hyphae passing through the intercellular space (Fig. 1F). In some epidermal cells, fungal pegs could be seen (Fig. 1G). The emanating hyphae are 2 to 5 μm in diam with clamp connections. The inner plan view of the mantle shows a net synenchyma with distinctly elongated cells (Fig. 1H).

Mycorrhiza of *Fagus crenata* The mycorrhiza of *F. crenata* is branched-monopodial, yellow when young and brownish yellow with age (Fig. 2A). The emanating hyphae are 2 to 5 μm in diam with clamp connections. The transect shows a dense mantle 30 to 40 μm thick and Hartig net penetrating deep into the cortical cell layer (Fig. 2B). The inner plan view of the mantle again shows a net synenchyma (Fig. 2C). In the outer cortical cells, intracellular hypha can be seen (Fig. 2D).

Discussion

Björkman (1960) reported that rapid development of *Monotropa* occurred late summer, when the production of mycorrhizae reached its optimum, in contrast to the observations of *Monotropa* by Duddridge and Read

(1982) and of *M. globosum* in this study, in which growth was seen mainly in the early summer.

Monotropastrum globosum presents a thick sheath and reduced Hartig net (Fig. 1E), similar to *Monotropa hypopitys* Linn. (Björkman, 1960), *Pterospira andromedea* Nuttall and *Sarcodes sanguinea* Torrey (Robertson and Robertson, 1982). Another structure observed in *M. globosum* mycorrhizae is the fungal peg (Fig. 1G). This haustoria-like structure found in the epidermal cells as a fungal intrusion was also observed in the ectendomycorrhiza of *S. sanguinea* (Robertson and Robertson, 1982), and in *Monotropa* by Melin, as cited by Björkman (1960). The formation of this structure is described in detail by Duddridge and Read (1982). They verified that this structure occurred mainly in early summer, the period of expansion of the flowering organs, and was believed to facilitate enhanced nutrient transfer.

Björkman (1960) isolated the fungus from *M. hypopitys* mycorrhizae, and inoculated it into *M. hypopitys* and conifers. He observed the formation of thick sheaths for *M. hypopitys* and coniferous mycorrhizae, but with reduced Hartig nets in the former and deep penetration into the cortex in the latter. Similar characteristics were observed in this study, where both *M. globosum* and *F. crenata* presented thick sheaths, but the former Hartig net was reduced whereas the latter was deep (Figs. 1E, 2B).

The fact that the *M. globosum* root system interpenetrates the *F. crenata* root system (Fig. 1B), together with the similarities in emanating hypha morphology be-

tween both systems, and the fact that the mantle inner view structures of both are net synenchyma (Figs. 1H, 2C), increase the probability that both mycorrhizae are formed by the same fungus. The mantle plan view structure, when examined microscopically, seems to be largely host-independent, as suggested by Ingleby et al. (1990).

The intracellular hypha in the cortical cells of *F. crenata* (Fig. 2D) is indicative of an ectendomycorrhiza. Ectendomycorrhizae have been verified in Pinaceae (Froidevaux and Amiet, 1975; Yang and Wilcox, 1984; Piché et al., 1986; Wilcox and Wang, 1987), *Arbutus menziesii* Prursh. (Zak, 1974), *Betula alleghaniensis* Britt. (Wilcox and Wang, 1987) and dipterocarps (Louis, 1988).

This study demonstrated that the fungus involved in this "epiparasitic" association formed ectendomycorrhizae with both *M. globosum* and *F. crenata*. Furthermore, the mycorrhiza formed by the former showed much evidence to justify its classification as "monotro- poid", as suggested by Duddridge and Read (1982) and Robertson and Robertson (1982).

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