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SHORT COMMUNICATION

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Difference in monotropoid mycorrhiza formation between *Monotropastrum globosum* and its forma *roseum*

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Abstract Monotropoid mycorrhiza formation and its morphological characteristics on an achlorophyllous plant, *Monotropastrum globosum* f. *roseum*, were investigated. The forma formed typical monotropoid mycorrhizae, indicating that the forma is myco-heterotrophic. Morphological characteristics of the mycorrhizae and root systems of the forma were quite different from those of *Monotropastrum globosum*, suggesting that further investigation on host specificity and the phylogeny of the plants should be conducted.

Key words *Monotropastrum* · Monotropoid mycorrhiza · Myco-heterotroph · Red tepal

Monotropastrum globosum H. Andres is an achlorophyllous herb and is considered to be myco-heterotrophic (obtains carbon from associated ectomycorrhizal fungi; see Leake 1994). The root system of the plant is called a root-ball (Smith and Read 1997), and its root tips form ectendomycorrhizae called monotropoid mycorrhizae (defined by Duddridge and Read 1982). Final hosts of the plants are thought to be ectomycorrhizal trees (Smith and Read 1997). The genus consists of one species, and distribution of the genus *Monotropastrum* is limited in Asia from the Himalayas to Japan (Satake et al. 1982). The species of this genus has been only slightly studied (Kasuya et al. 1995; Nakamura 1978; Tsukaya 1988), and only Kasuya et al. (1995) made detailed observations on mycorrhizal formation of *M. globosum*.

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Monotropastrum globosum H. Andres f. *roseum* Honda (Benibanaginryousou in Japanese; Fig. 1A) is distinguished from the mother species by its typical red tepals (Fig. 1B). This forma has been reported only from Miyazaki Prefecture, Kyusyu, Japan (Ogura 1995). Honda (1957) merely described the color of the flower but not the other characteristics of flower, roots, and mycorrhizae, although mycorrhizal formation is an important characteristic of achlorophyllous myco-heterotrophic species. This article reports mycorrhizal formation of the forma and compares the root system and morphology of mycorrhizae between the forma and *M. globosum*.

We made samplings twice, on July 5, 2000 and June 26, 2001. We collected five individuals of the forma per sampling date. Study site of the forma was along a trekking road of Mt. Kirishima, Makizono-cho, Kagoshima Prefecture (31°55′ N, 130°50′ 30″ E; altitude, 1100 m). *Pinus densiflora* Sieb. et Zucc., *Abies firma* Sieb. et Zucc., and *Tsuga Sieboldii* Carr. were dominant in the forest. *Quercus mongolica* Fischer ex Turcz. var. *grosseserrata* (Bl.) Rehd. et Wils. was also observed in the canopy layer at higher altitude. All these trees are ectomycorrhizal. *Symplocos myrtacea* Sieb. et Zucc., *Carpinus laxiflora* (Sieb. et Zucc.) Blume, and *Cinnamomum insularimontanum* Hayata, among others, were distributed in the subcanopy layer. For comparison, we also collected a typical individual of *M. globosum* on June 9, 2000, at Mt. Hikagedaira, Gifu Prefecture (36°8′30″ N, 137°27′ E; altitude, 1500 m), where *Q. mongolica* var. *grosseserrata* and *Betula platyphylla* Sukatchev var. *japonica* (Miq.) Hara (both are ectomycorrhizal) were dominant. The plant nomenclature is based on Kitamura and Murata (1979a,b).

Under the binocular, root systems and mycorrhizal formation were observed. Ten root tips per individual were detached from each root-ball and were cross sectioned by hand. All the preparations were observed under a light microscope. Morphological typing of the mycorrhizae by mantle structures was also examined according to Ingleby et al. (1990) on three whole root mounts from each individual.

The forma *roseum* formed a root-ball (Fig. 2A) different from that of *M. globosum* (Fig. 2B). The root-ball of the

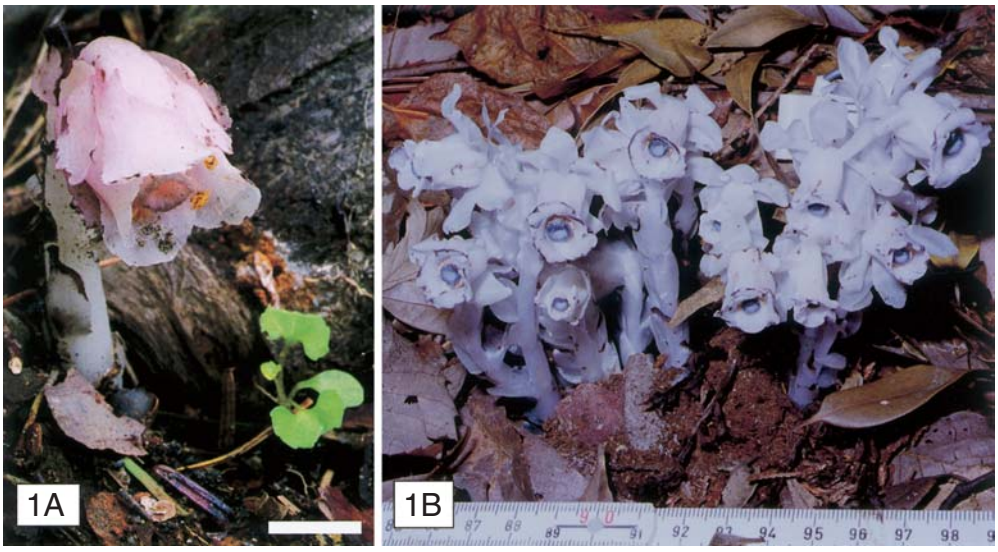

Fig. 1. Flowers of achlorophyllous plants, *Monotropastrum globosum* f. *roseum* and *M. globosum*. **A** Flower of *M. globosum* f. *roseum* has red-colored tepals. **B** Flowers of *M. globosum* with white tepals. *Bar* 1 cm

forma did not seem to be part of the plant but a clod (Fig. 2A), and each root tip was indistinct (Fig. 3A). In contrast, a root-ball of *M. globosum* (Fig. 2B) was easily recognized, and each root tip was quite distinct (Fig. 3B).

Mycorrhizal formation was easily recognizable on the forma because a white-colored mantle was evident (Fig. 3A). Under the light microscope, all the root tips of the forma were found to form typical monotropoid mycorrhizae, which had a Hartig net and fungal pegs involving the first layer of the plant cortical cells (Fig. 4A). All the tips of the forma were judged to be morphologically the same: the mantle was thinner than 20 μm; the hyphal arrangement of mantle was felt prosenchyma to net prosenchyma; and emanating hyphae, often with a knoblike tip, were frequently found, but no clamp connections or cystidia were found (Fig. 5A), which were different from those of *M. globosum* as described next.

Mycorrhizal formation of *M. globosum* was also easily recognizable by observation of the surface of each root tip (Fig. 3B), and all of these were monotropoid mycorrhizae (Fig. 4B). The mantle was about 30 μm thick; the hyphal arrangement of the mantle was net synenchyma; and emanating hyphae were rarely found; these observations were very similar to the observation by Kasuya et al. (1995), although no clamp connections were found in this study (Fig. 5B).

M. globosum f. *roseum* was found to form monotropoid mycorrhizae, suggesting that the forma is also a mycoheterotrophic plant as are other monotropoid plants (Leake 1994). However, the mycorrhiza of the forma was different from that of *M. globosum*, and also differed from observations by Kasuya et al. (1995). Ingleby et al. (1990) reported that a particular mycorrhizal structure corresponds to one fungal species; therefore, this forma is thought to have a different fungal host from that of *M. globosum*, although the species name of the host fungus was obscure in this study. Also the host may be one fungal species because all the tips in this study were morphologically the same.

Bidartondo and Bruns (2001) suggested that the difference in fungal host specificity of monotropoid plants reflects the phylogenetic difference of the plants. Thus, the differences in the mycorrhizal characteristics between the forma and *M. globosum* indicate that the two plants may speciate sufficiently as their fungal hosts do not overlap each other. Moreover, the flowering seasons of the two plants do not overlap (Kurogi, unpublished data), which is consistent with this point of view. To further clarify the speciation of *M. globosum* f. *roseum* from the mother species, identification and comparison on fungal hosts between these plants and phylogenetic studies on the plants with molecular techniques in neighboring habitats are needed.

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References

Bidartondo MI, Bruns TD (2001) Extreme specificity in epiparasitic Monotropoideae (Ericaceae): widespread phylogenetic and geographical structure. Mol Ecol 10:2285–2295

Duddridge JA, Read DJ (1982) An ultrastructual analysis of the development of mycorrhizas in *Monotropa hypopitys* L. New Phytol 92:203–214

Honda M (1957) Scientific names of plants of Japan (in Japanese). Kousei-sya, Tokyo

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. Institute of Terrestrial Ecology, Natural Environmental Council, London

Kitamura S, Murata G (1979a) Coloured illustrations of woody plants of Japan. I (in Japanese). Hoikusya, Osaka

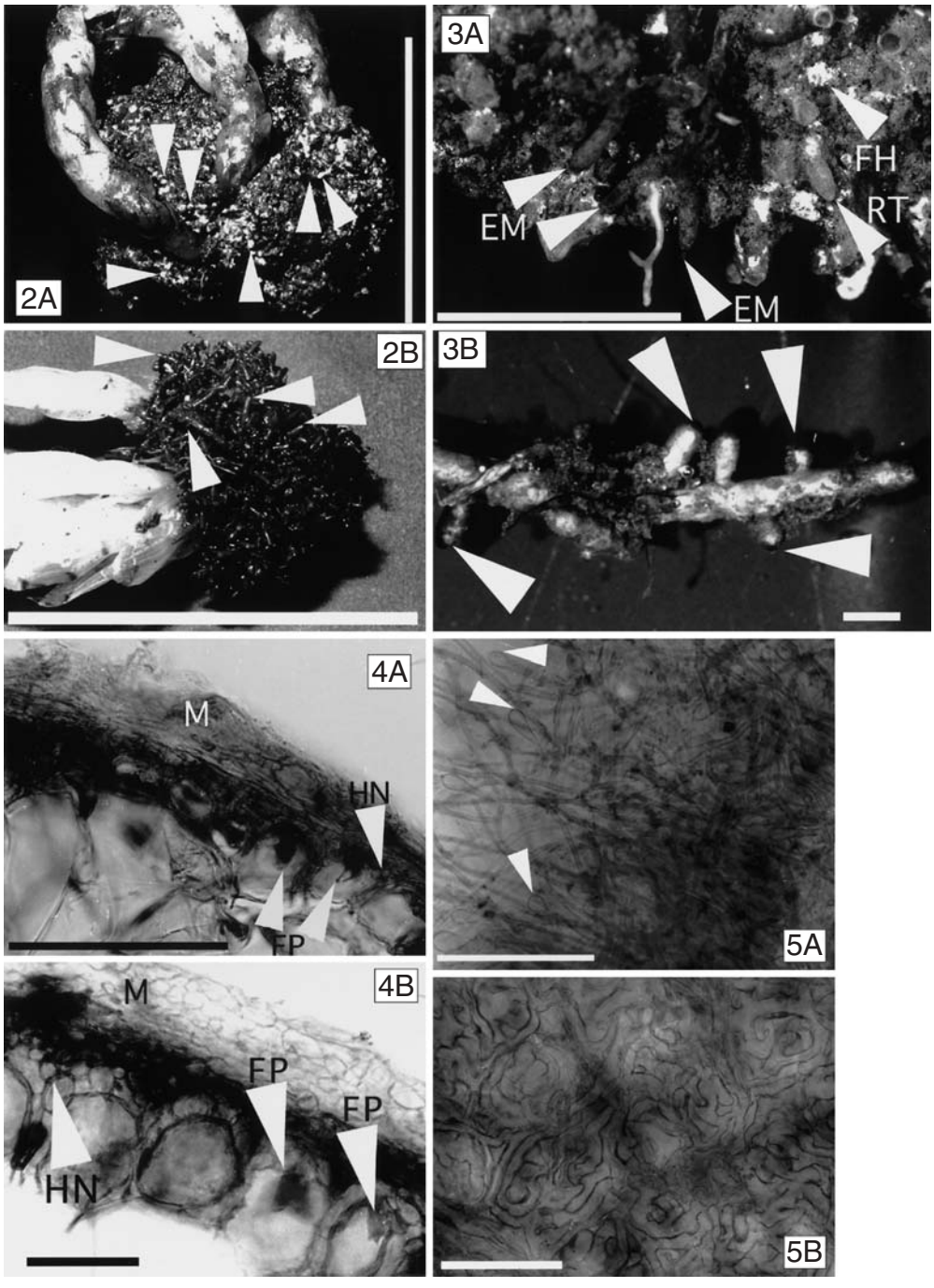

Fig. 2. Root-balls of the two plants. **A** *M. globosum* f. *roseum*. Branching of roots is not apparent. **B** *M. globosum*. Root tips and their branching are easily recognizable. *Arrowheads*, apex of root tips. *Bars* 5 cm

Fig. 3. Magnification of the root-ball of each plant. **A** *M. globosum* f. *roseum*. Root tips are not apparent, but fungal hyphae are visible. Ectomycorrhizae of a Pinaceae plant are also seen, but they are thought to be old and senescent because of their darkened color. **B** *M. globosum*. *Arrowheads*, root tip of monotropoid mycorrhizae; *EM*, ectomycorrhiza; *FH*, fungal hyphae; *RT*, root tip of the forma *roseum*. *Bars* **A** 5 mm; **B** 1 mm

Fig. 4. Cross sections of the root tip of the two plants stained with trypan blue. **A** *M. globosum* f. *roseum*. Emanating hyphae from the mantle are apparent. The Hartig net and many fungal pegs involve the first layer of the plant cortical cells. **B** *M. globosum*. Emanating hyphae are not apparent. The Hartig net and many fungal pegs involve the first layer of plant cortical cells. *FP*, fungal peg; *HN*, Hartig net; *M*, mantle. *Bars* **A** 50 μm; **B** 30 μm

Fig. 5. Mantle structure of the two plants stained with trypan blue. **A** *M. globosum* f. *roseum*. Hyphal arrangement of the mantle was felt prosenchyma to net prosenchyma. Emanating hyphae, often with a knoblike tip, were frequently found but no clamp connections or cystidia were found. **B** *M. globosum*. Hyphal arrangement of the mantle was net synenchyma. Emanating hyphae were rarely found; no clamp connections or cystidia were found. *Arrowheads*, knoblike tip of emanating hypha. *Bars* 30 μm

Kasuya MCM, Masaka K, Igarashi T (1995) Mycorrhizae of *Monotropastrum globosum* growing in a *Fagus crenata* forest. Mycoscience 36:461–464

Kitamura S, Murata G (1979b) Colored illustrations of woody plants of Japan. II (in Japanese). Hoikusya, Osaka

Leake JR (1994) Transley review no. 69: the biology of myco-heterotrophic ('saprophytic') plants. New Phytol 127:171–216

Nakamura S (1978) Can the holomycotrophyte be cultured without fungal symbionts? A gnotobiological view (in Japanese). Trans Mycol Soc Jpn 19:325–331

Ogura I (1995) Plants around Miyakonojo Basin (in Japanese). Kyoei Print, Miyazaki, Japan

Satake Y, Ohwi J, Kitamura S, Watari S, Tominari T (1981) Wild flowers of Japan. Herbaceous plants III (in Japanese). Heibonsya, Tokyo

Smith SE, Read DJ (1997) Arbutoid and monotropoid mycorrhizas. In: Mycorrhizal symbiosis, 2nd edn. Academic Press, London, pp 301–322

Tsukaya H (1988) Saprophagy of Monotropaceae and its mycorrhizal fungi (in Japanese). Nippon Kingakkai Nyûsu 11:41–46