

## ノート (Note)

# The ectomycorrhizal basidiomycete *Tricholoma matsutake* associates with the root tissues of the model tree *Populus tremula* × *tremuloides* in vitro

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*Tricholoma matsutake* is an ectomycorrhizal basidiomycete that produces the prized mushroom “matsutake” in association with the Pinaceae (Yamada et al. 2014). Since *Populus* associates with a wide range of ectomycorrhizal fungi (Kaldorf et al. 2002, Krpata et al. 2008), we hypothesized that *T. matsutake* could associate with *Populus tremula* × *tremuloides*, a model tree species that is widely used for the elucidation of tree physiology, especially in the field of biochemistry, genetics and molecular biology (Jansson and Douglas 2007). In the present study, we demonstrated that *T. matsutake* predominantly exhibited an endophytic association with the roots of *P. tremula* × *tremuloides*, in which hyphae colonized intercellular spaces that paralleled the vascular bundles, while it sporadically displayed ectomycorrhizae by forming a Hartig net around the epidermal layer.

*Populus tremula* × *tremuloides* Pt-T6 (Jansson and Douglas 2007) was cultured in the presence and absence of *T. matsutake* Y1 (= ATCC MYA-915, NBRC 33136) on a granite-based soil substrate (Murata et al. 2015). One hundred sixty days after inoculation, the roots of the plants in both cultures were

examined under a microscope (Murata et al. 2015), and their dry weights were analyzed by the Student’s *t*-test ( $P < 0.05$ ). The experiment was performed twice independently under the same culture conditions, except specimens from the second experiment were temporarily placed in a different facility than that of the first for ca. one-third of the incubation period.

In the first experiment, *P. tremula* × *tremuloides* that associated with *T. matsutake* formed two types of roots (Fig. 1A), one having dark brown ectomycorrhiza-like root tips (Fig. 1B) and the other exhibiting non-ectomycorrhiza-like root tips without mycelial colonization (Fig. 1C); the former occurred at a much lower frequency than the latter, which dominated the root system (Fig. 1A). Note that roots that were cultured without the fungus were transparent gray and exhibited root hairs (Fig. 1D). The fungal mycelia cultured without plants did not grow over the substrate, and the inoculum remained at the inoculation site. In sections of live, dark brown, lateral roots of *P. tremula* × *tremuloides* that were associated with *T. matsutake*, a fungal sheath covered the epidermis and a Hartig net formed around the epidermal layer (Fig. 2A, B). In contrast, in sections of live,

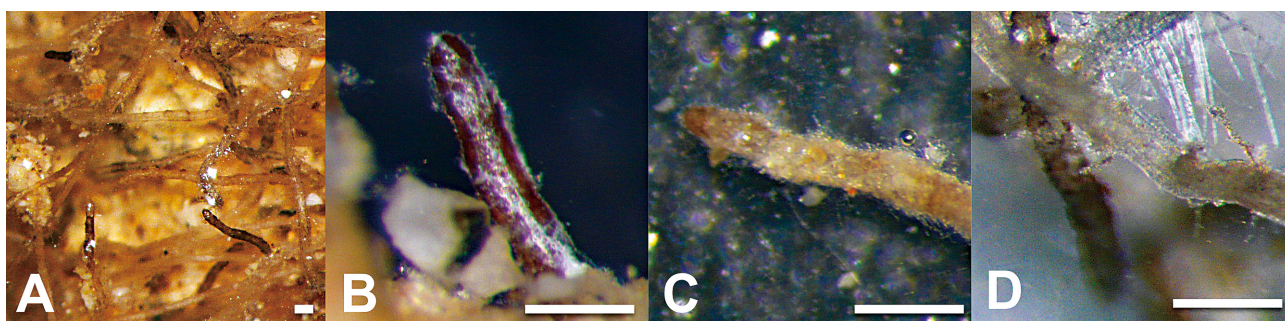


Fig. 1. Dissecting micrograph of the root systems of *Populus tremula* × *tremuloides* colonized by mycelia of *Tricholoma matsutake*. (A) Two types of *P. tremula* × *tremuloides* roots (dark brown and light brown) associated with *T. matsutake*. (B) Ectomycorrhiza formed between *P. tremula* × *tremuloides* and *T. matsutake*. (C) Root of root endophytic association between *P. tremula* × *tremuloides* and *T. matsutake*. (D) Roots of *P. tremula* × *tremuloides* without *T. matsutake*. Scale bars 1 mm.

外生菌根性担子菌マツタケと実験樹種 *Populus tremula* × *tremuloides* との組織内における in vitro での相互作用  
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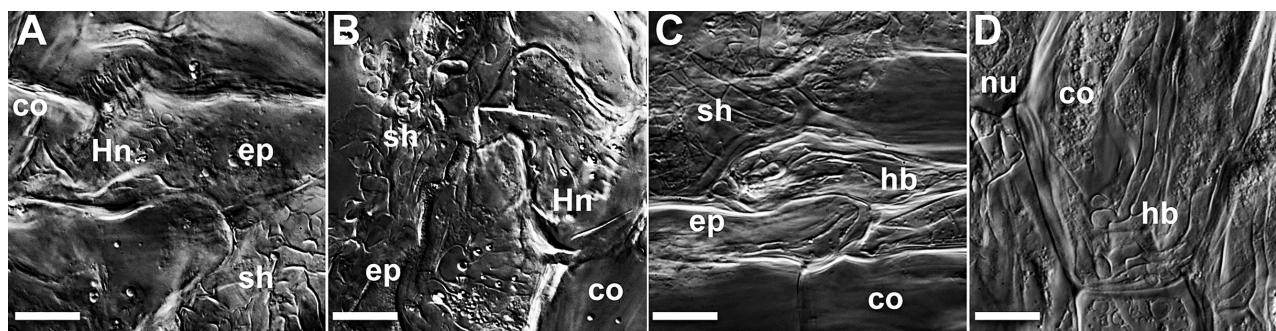


Fig. 2. Differential interference contrast Nomarski micrographs of ectomycorrhizae formed between *Populus tremula* × *tremuloides* and *Tricholoma matsutake*. (A) Cross-section of the Hartig net. (B) Longitudinal section of the Hartig net. (C–D) Longitudinal section of the root endophyte association at the epidermal layer. Abbreviations: *co* cortical cell, *ep* epidermis, *hb* hyphal bundle, *Hn* Hartig net, *sh* mycelial sheath. Scale bars 10 μm.

Table 1. Parameters related to growth of *Populus tremula* × *tremuloides* in association with *Tricholoma matsutake*.

Measured parameter	1 <sup>st</sup> -ex Mean (SE) <sup>a</sup>		2 <sup>nd</sup> -ex Mean (SE) <sup>b</sup>		Significance probability <sup>c</sup>	
	+ Tm	- Tm	+ Tm	- Tm	1 <sup>st</sup> -ex	2 <sup>nd</sup> -ex
Above ground weight (mg)	502.4 (33.5)	287.1 (59.6)	488.9 (48.7)	387.1 (41.0)	0.0109	0.0398
Below ground weight	129.2 (21.1)	70.3 (6.4)	179.0 (37.6)	64.4 (8.9)	0.04814	0.0030
Total dry weight (mg)	631.6 (46.9)	357.4 (59.4)	667.9 (74.3)	451.5 (44.2)	0.00787	0.0037

<sup>a</sup> N=5 for specimens with *T. matsutake* indicated by “+Tm”. N=4 for specimens without *T. matsutake* as indicated by “-Tm”. 1<sup>st</sup>-ex = the first experiment.

<sup>b</sup> N=6 for specimens with and without *T. matsutake*. 2<sup>nd</sup>-ex = the second experiment.

<sup>c</sup> Significant differences ( $P < 0.05$ ) between treatments with and without *T. matsutake* were determined using Student's t-test.

light brown, lateral roots of the hybrid aspen that associated with the fungus, there were root endophytic associations, in which the fungal hyphae penetrated the intercellular spaces of the epidermal and cortical cell layers (Fig. 2C); some hyphae were apparently localized inside cortical cells (Fig. 2D). However, in the second co-culture experiment, all the fungi exhibited an endophytic association with the plant roots, and ectomycorrhizae were not found. In both cases, plant growth was significantly promoted by association with the fungus, suggesting that a symbiosis was established (Table 1). The reason for the variation in ectomycorrhizae formation between the two experiments needs to be clarified. Further analysis is required to determine the feasibility of using *P. tremula* × *tremuloides* as a host for in vitro *T. matsutake* research.

#### Acknowledgments

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