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SHORT COMMUNICATION

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Ectomycorrhizal and arbuscular mycorrhizal colonization of two species of floodplain willows

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Abstract To understand the relationships between the distribution of *Chosenia arbutifolia* and *Salix sachalinensis* and their mycorrhizal colonization, changes in the quality and types of ectomycorrhizas and arbuscular mycorrhizas of the seedlings of two species were studied at five different sites with different soil conditions in the floodplain of the Satsunai River, Hokkaido. High ectomycorrhizal and low arbuscular mycorrhizal colonization were found in roots of both plants. Ectomycorrhizal colonization of *S. sachalinensis* in wet sandy or muddy soil conditions was at the same level as that in dry gravelly sites. In contrast, ectomycorrhizal colonization of *C. arbutifolia* seedlings was lower from wet sandy sites than that from dry gravelly sites. In all study sites, the same three morphological types of ectomycorrhizas were dominant.

Key words Arbuscular mycorrhiza · *Chosenia arbutifolia* · Ectomycorrhiza · Floodplain · *Salix sachalinensis*

Mycorrhizal fungi form mutualistic associations with plant roots. The important roles of mycorrhizal fungi in promoting survival and growth of seedlings by enhancing tolerance to drought, shading, or disease, and by increasing uptake of nutrients or water, have been demonstrated previously (Marx 1969; Dixon et al. 1984; Abuzinadah and Read 1989; MacFall et al. 1991; Browning and Whitney 1993; Hashimoto and Hyakumachi 2000, 2001). Thus, mycorrhizal symbiosis is important for newly established pioneer plant seedlings.

On the floodplain of the Satsunai River, many willow (salicaceous) species such as *Chosenia arbutifolia* (Pall.) A. Skvortz, *Salix sachalinensis* Fr. Schm., *S. rorida* Lackschewiz, *S. gracilistyla* Miq., *S. integra* Thunb. *S. pet-susu* Kimura, and *Populus maximowiczii* Henry are found. Among these salicaceous species, *C. arbutifolia* is a relict species that is known to be found in only two locations in Japan: Azusa River, central Honshu, and the rivers in the Tokachi Plain and Shokotsu River in Hokkaido (Tatewaki 1948; Kimura 1951, 1952). In the floodplain of the Satsunai River, *C.* a*rbutifolia* predominated on dry gravelly soil, whereas *Salix* species were dominant on wet sandy soil (Ishikawa 1987; Niyama 1989). Although the mature trees of *C. arbutifolia* and *Salix* species are not found in the same sites, their seedlings (1–3 or 4 years old) were common on both the dry gravelly soil and wet sandy soil sites in the floodplain.

Some species of *Salix* are found to form both arbuscular mycorrhizas and ectomycorrhizas (Lodge 1989; Dhillion 1994; Smith and Read 1997; van der Heijden et al. 1999; van der Heijden and Vosatka 1999; van der Heijden 2001; Sasaki et al. 2001). The difference of ectomycorrhizal and arbuscular mycorrhizal colonization patterns of *C. arbutifolia* and *Salix* species might affect their ability to become established on the wet soil of the floodplain. However, there are only a few studies on the relationship between the two types of mycorrhizal colonization of *Salix* species and soil conditions (Lodge 1989; van der Heijden et al. 1999; van der Heijden and Vosatka 1999), and no research on mycorrhizas of *Chosenia* species has been found.

The purpose of this study is to understand the relationship between the distribution of *C. arbutifolia* and *S. sachalinensis* and their mycorrhizal colonization in several different soil conditions. The differences in the quality and types of ectomycorrhizal and arbuscular mycorrhizal colonization between *C. arbutifolia* and *S. sachalinensis* seedlings were examined at five different sites with different soil conditions in the floodplain of the Satsunai River, Hokkaido.

Seedling samples were taken from five sites (sites A, B, C, D, and E) located in the floodplain midstream of the Satsunai River (Fig. 1A). These sites were located in Obihiro City in Hokkaido Prefecture, Japan (42°52′ N,

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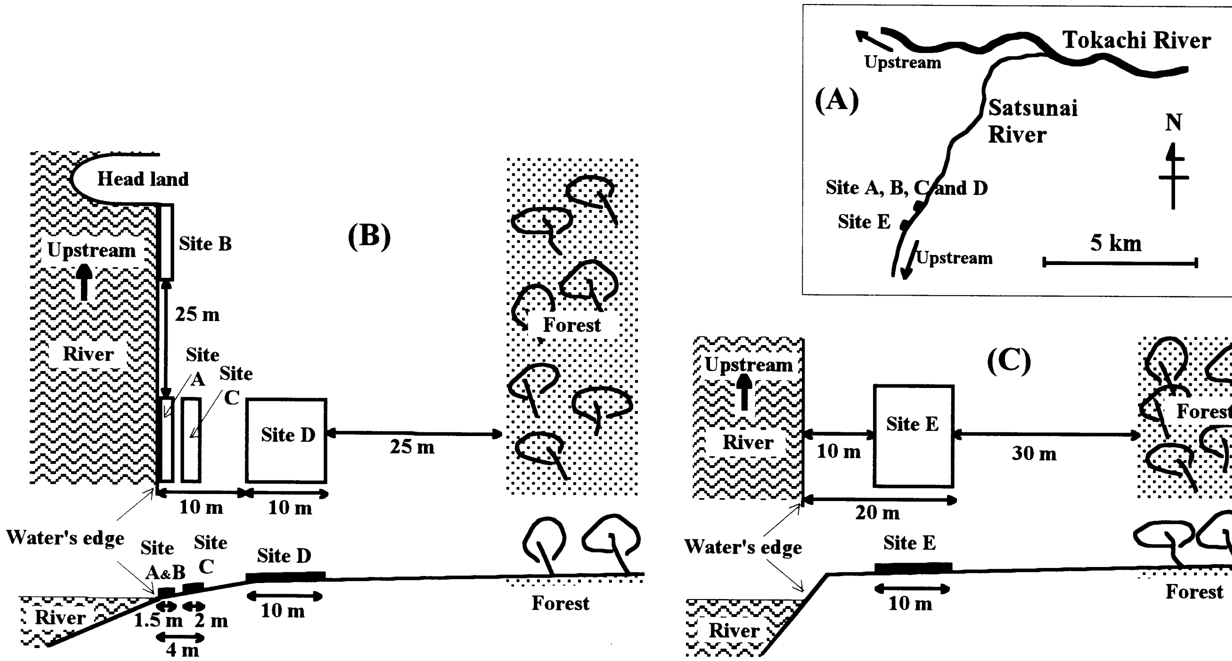

Fig. 1. Study area in the floodplain midstream of the Satsunai River. **A** Location of sites A, B, C, D, and E. **B**,**C** Layout and profile of sites A–E

Table 1. Characteristics of the five sites in the Satsunai River floodplain

Site	Distance to the water's edge (m)	Soil moisture[a] (%)	Soil property	Dominant salicaceous species
A	0–1.5	Wet (25.2)	Sandy	*Chosenia arbutifolia, Salix sachalinensis, Salix pet-susu*
B	0–1.5	Wet (34.2)	Muddy	*S. sachalinensis, S. pet-susu*
C	2–4	Medium wet (14.5)	Sandy	*C. arbutifolia, S. sachalinensis, S. pet-susu*
D	10–20	Dry (6.2)	Gravelly	*C. arbutifolia*
E	10–20	Dry (7.2)	Gravelly	*C. arbutifolia, S. sachalinensis, S. pet-susu*

[a] Water content by weight on October 10, 2001

143°12′ E), where the annual mean temperature is about 6.0°C, annual rainfall is 885 mm, and maximum snow depth is about 94 cm. The distance to the average level of the water's edge was 0–1.5, 0–1.5, 2–4, 10–20, and 10–20 m in sites A, B, C, D, and E, respectively (Fig. 1B,C). The soil conditions were wet sandy, wet muddy, medium wet sandy, dry gravelly, and dry gravelly in sites A, B, C, D, and E, respectively (Table 1). The seedlings of *C. arbutifolia* were not found in site B and those of *S. sachalinensis* did not occur in site D. The other sites had both naturally regenerated *C. arbutifolia* and *S. sachalinensis* seedlings. *Artemisia montana* (Nakai) Pamp. and *Silene armeria* L. were the most common herbaceous species in those sites. Descriptions and soil conditions of the five sampling sites are shown in Table 1.

Entire root systems of the seedlings were gently grubbed out using a trowel and brush to avoid damaging the fine roots. Seven seedling samples of *C. arbutifolia* and *Salix sachalinensis* were collected from each of the five sites on June 9, July 24, and September 5, 2001. Length of the main stem of the sample seedlings was 5–15 cm. Age of the seedlings, as confirmed by annual rings, was 2–4 years. Most of the root systems of the sample seedlings had well-developed lateral roots. After the sampling at September, the sampling sites were flooded after heavy rains from a typhoon. As this flooding caused damage to the vegetation in sites A, B, and C, sampling was not carried out thereafter.

The seedlings were collected from each site, gently washed to remove soil particles, and soaked in distilled water. The entire root system of each seedling was evaluated for the occurrence of ectomycorrhizas. An ectomycorrhiza was confirmed by the presence of a fungal mantle on the root tip using a compound light microscope (400×–1000×). Ectomycorrhizal types were distinguished macroscopically (8×–40×) and microscopically (400×–1000×) by reference to morphological characteristics such as color, size, shape, and surface features of mantle tissues by the method of Ingleby et al. (1990). The numbers of total root and ectomycorrhizal root tips were counted, and the lengths of total root and ectomycorrhizal root were measured under a stereomicroscope (8×–40×) using the method of Tennant (1975). Together with sampling for ectomycorrhizal colonization, four seedling root samples each for arbuscular mycorrhizal colonization were made on June 9 and September 5 and stored in 70% alcohol until processed. Samples were immersed in water over a 1-mm-mesh sieve to remove the soil particles and rinsed gently. Clean root samples were cleared in 10% KOH solution for 10 min at 121°C and 10 min in 10% H_2O_2 at room temperature, stained with 0.05% chlorazole black E solution at 90°C for 90 min, and then stored in glycerol solution. Colonization was evaluated according to the methods of McGonigle et al. (1990).

Chosenia arbutifolia and *S. sachalinensis* seedlings had ectomycorrhizal roots in the samples from all five sites (Table 2). The percentages of ectomycorrhizal colonization of seedlings increased with the sampling periods. Ectomycorrhizal colonization of *C. arbutifolia* seedlings from wet sandy soil (site A) were lower than that from the other three sites with dry conditions (sites C, D, and E). There were no significant differences between the ectomycorrhizal colonization of *S. sachalinensis* seedlings from wet sandy and muddy sites (sites A and B) and from the other two sites (sites C and E) except for site A on June 9. In the wet sandy soil (site A), the percent ectomycorrhizal root length of *C. arbutifolia* seedlings on June 9, July 24, and September 5 (0.3%, 2.9%, and 23.7%, respectively) was lower than that of *S. sachalinensis* seedlings (9.3%, 19.3%, and 36.2%, respectively).

Table 2. Percentage of ectomycorrhizal roots in *Chosenia arbutifolia* and *Salix sachalinensis* seedlings collected from five different sites of the Satsunai River floodplain

Site	June 9	July 24	September 5
Chosenia arbutifolia			
A	0.3 ± 0.3 a	2.9 ± 1.1 a	23.7 ± 4.5 a
B	–	–	–
C	11.7 ± 1.5 b	21.4 ± 2.1 b	34.2 ± 4.2 ab
D	10.8 ± 3.7 b	19.0 ± 3.6 b	36.3 ± 4.7 ab
E	16.4 ± 3.7 b	22.9 ± 3.9 b	48.9 ± 6.1 b
Salix sachalinensis			
A	9.3 ± 2.4 a	19.3 ± 4.4 a	36.2 ± 2.6 a
B	13.8 ± 3.2 ab	13.8 ± 3.2 a	38.6 ± 1.2 a
C	12.5 ± 2.7 ab	25.0 ± 7.6 a	42.2 ± 2.9 a
D	–	–	–
E	18.3 ± 2.4 b	22.7 ± 3.1 a	39.8 ± 1.9 a

Data (mean ± SD) are presented as percentages of total root length
Within a column, data followed by the same letter are not significantly different ($P = 0.05$) from each other according to the least significant difference multiple range test
–, absence of *C. arbutifolia* or *S. sachalinensis* seedlings ($n = 7$)

Four different morphological types (types 1–4) of ectomycorrhizas were found among the five sites. Types 1, 2, and 3 were observed on both *C. arbutifolia* and *S. sachalinensis* seedlings from all five sites. Type 1 was the most abundant, associated with the seedling roots of both species collected from all five sites. Average percentage occurrence of type 1 of *C. arbutifolia* and *S. sachalinensis* seedlings was 66.9% and 61.8%, respectively; this type was macroscopically characterized by beige to pale buff color with age and fairly straight, infrequently short-branched hyphae (Fig. 2a). Soil particles are particularly difficult to remove from these sheath surfaces. Type 1 was microscopically found to have torturous emanating hyphae bearing clamp connections and a net synenchyma sheath (Fig. 2d). Type 2 was characterized by silver-white color when young, changing to fawn color with age, and fairly straight, slender, and infrequently short-branched hyphae (Fig. 2b). Type 2 had emanating hyphae bearing prominent clamp connections and a felt prosenchyma (surface) (Fig. 2e) and net synenchyma (inner) sheath. The average percentage occurrence of type 2 in *C. arbutifolia* and *S. sachalinensis* seed-

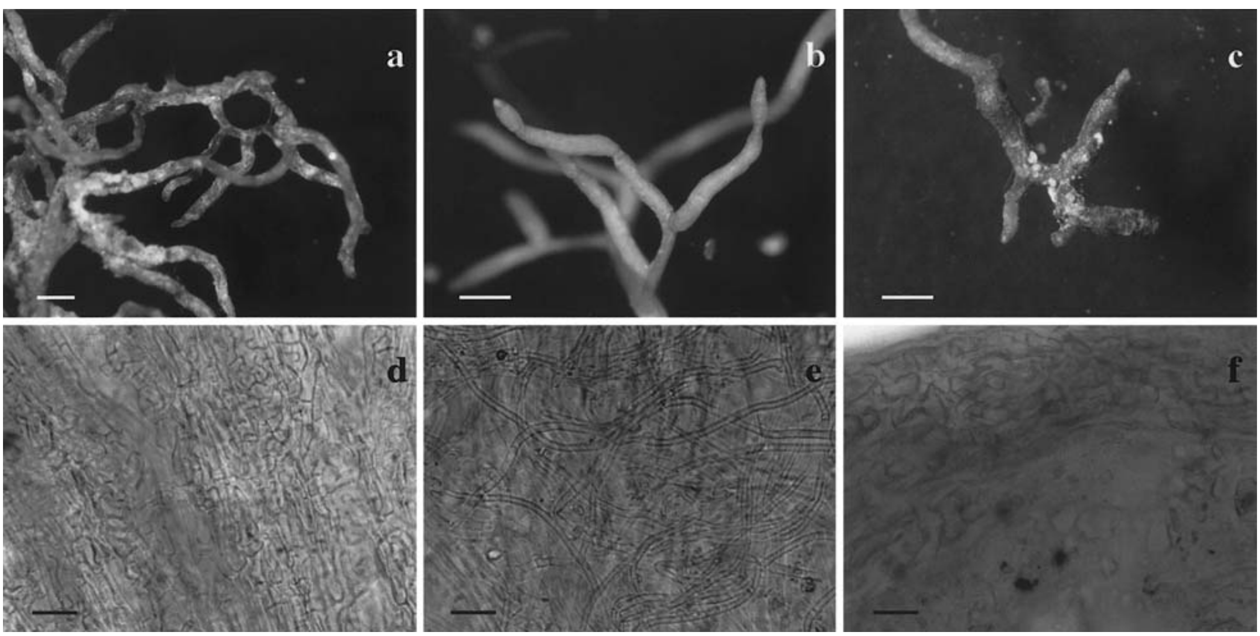

Fig. 2. Ectomycorrhizas in *Chosenia arbutifolia* and *Salix sachalinensis* seedlings collected from the Satsunai River floodplain. **a** Type 1 was the dominant ectomycorrhizal type (>60%). **b**,**c** Types 2 and 3, respectively. **d**,**e**,**f** Microscopic sheath surface of types 1, 2, and 3, respectively. *Bars* **a**–**c** 5 mm; **d**–**f** 10 μm

Table 3. Percentage of arbuscular mycorrhizal roots in *Chosenia arbutifolia* and *Salix sachalinensis* seedlings collected from five different sites of the Satsunai River floodplain (Mean ± SD)

Site	June 9	September 5
Chosenia arbutifolia		
A	0	0.83 ± 0.57
B	–	–
C	0.56 ± 0.65	0.21 ± 0.24
D	0	0
E	0	0.79 ± 0.59
Salix sachalinensis		
A	0	0.18 ± 0.20
B	0.21 ± 0.24	0.99 ± 0.92
C	0.25 ± 0.29	0.14 ± 0.16
D	–	–
E	0	0

–, absence of *C. arbutifolia* or *S. sachalinensis* seedlings (n = 4)

lings was 20.9% and 16.1%, respectively. Macroscopic features of type 3 were brown to dark brown in color, and stubby mycorrhizas with an irregular branching pattern (Fig. 2c). Soil particles are also difficult to remove from these sheath surfaces. This type showed torturous emanating hyphae with no clamp connections and an irregular synenchyma sheath surface under the microscope (Fig. 2f). The average percentage occurrence of type 3 in *C. arbutifolia* and *S. sachalinensis* seedlings was 12.5% and 21.6%, respectively. Type 4 was characterized by abundant, coarse, dark brown to black emanating hyphae. This type was identified as *Cenococcum geophilum* Fr. by its black color, projecting hyphae, and pseudoparenchymatous sheath (Pigott 1982). This type was observed in only one *S. sachalinensis* seedling sample of July 24 at site C. There was no correlation between the occurrence of these four morphological types of ectomycorrhizas and host plant species or conditions of the sites.

The percentages of arbuscular mycorrhizal roots of *C. arbutifolia* and *S. sachalinensis* seedlings from five different sites are shown in Table 3. Arbuscular mycorrhizal colonization was less than 1% in all cases.

In comparison between arbuscular mycorrhizal and ectomycorrhizal colonization of *C. arbutifolia* and *S. sachalinensis* seedlings, arbuscular mycorrhizal colonization was always at very low levels (<1%) in all five different sites (Tables 2, 3). Helm et al. (1996) suggested that mycorrhizal communities at Exit Glacier were dominated by ectomycorrhizal fungi, even on the floodplain with mostly mineral soil, and that arbuscular mycorrhizal fungi were not found on any woody plants, including two *Salix* species. In dune ecosystems, high ectomycorrhizal and low arbuscular mycorrhizal colonization of *Salix repens* was shown at 16 sites in different successional stages (van der Heijden and Vosatka 1999). Sasaki et al. (2001) showed that the colonization level of arbuscular mycorrhizas of *Salix gracilistyla* seedlings in a nutrient-poor fluvial bar was relatively low. Thus, in barren soil sites arbuscular mycorrhiza seems not to be common in *Salix* and *Chosenia* species.

Ectomycorrhizal colonization of *S. sachalinensis* from the wet sandy or muddy sites were at the same level as that of dry gravelly sites, whereas ectomycorrhizal colonization of *C. arbutifolia* seedlings from wet sandy soil was lower than that from the dry gravelly sites (see Table 2). Ishikawa (1987) and Niyama (1989) suggested that *C. arbutifolia* is adapted to the dry sandy and gravelly soil condition. *Chosenia arbutifolia* had a narrow niche and was segregated from the other salicaceous species (Niyama 1989). One of the reasons for this narrow niche of *C. arbutifolia* might be the low level of ectomycorrhizal colonization in the wet soil conditions.

In the present study, only four different morphological types of ectomycorrhizas were found from the total 168 seedlings. It seems that the diversity of ectomycorrhizal fungi in the floodplain of the Satsunai River is low. van der Heijden et al. (1999) found more than 15 morphological types of ectomycorrhizas from the roots of mature *S. repens* in coastal dune. The Satsunai River has always flooded frequently and there is a shortage of water, especially in summer. The unstable conditions of the floodplain of this river might be the reason for the low diversity of ectomycorrhiza of *C. arbutifolia* and *S. sachalinensis* seedlings.

In general, the soil properties and moisture conditions affect the ectomycorrhizal fungal community. Sites that are often flooded by the rise of a river might be inappropriate for mycorrhizal fungi because mycorrhizal fungi are aerobic (Slankis 1974). However, *C. arbutifolia* and *S. sachalinensis* seedlings had ectomycorrhizal roots even in the wet sandy or muddy sites (see Table 2). The dominant three ectomycorrhizal morphological types (types 1, 2, and 3) in these wet sandy or muddy sites were identical to those of the other dry gravelly sites. These type 1, 2, and 3 ectomycorrhizal fungi might be well suited to the floodplain that is frequently disturbed by flooding and had sandy gravelly conditions, and colonize with the establishment of *C. arbutifolia* and *S. sachalinensis* seedlings on these sites.

In the Satsunai River floodplain, *C. arbutifolia* predominated on dry gravelly soil whereas *Salix* species were dominant on the sandy soil (Ishikawa 1987; Niyama 1989). The present study suggests that ectomycorrhizal fungi may play an important role in the establishment of *C. arbutifolia* and *S. sachalinensis* seedlings at the initial stage of revegetation following the flooding disturbance. One of the reasons for habitat segregation of *C. arbutifolia* and *S. sachalinensis* might be the low acquisition of ectomycorrhizal colonization of *C. arbutifolia* at sites having wet condition.

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