# Fungal endorhizal associates of *Equisetum* species from Western and Arctic Canada.

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**Abbreviations**:

| | |
|---|---|
| AM | arbuscular mycorrhiza; |
| DSE | dark septate endophyte; |
| FE | fine endophyte; |
| MQM | multiple quantitation microintersect; |
| Ma | million years ago; |
| SE | septate endophyte |

**Abstract** We describe endorhizal fungi associated with *Equisetum* species collected from Ellesmere Island (82 ºN), Axel Heiberg Island (80 ºN), and from sites in Yukon Territory and the Prairie Provinces (67 ºN - 51 ºN). Fungal colonization was assessed using a multiple quantitation microintersect method for lactofuchsin-stained roots examined with wide-field and confocal epifluorescence microscopy. *Equisetum* roots host abundant and diverse endorhizal fungal associates. For 85 specimens from 14 sites, total colonization averaged 30 ± 3 %, range 0 - 97 %. Colonization rates for wide aseptate hyphae characteristic of arbuscular mycorrhizae (5 ± 1 %) was significantly less than for fine endophytes (20 ± 3 %) or septate endophytes (17 ± 2 %). *Equisetum* spp. are abundant in tundra and an important understory plant in boreal forests, where they are particularly common on burned or disturbed sites. Endorhizal fungi associated with *Equisetum* may have broad ecological relevance.

## Introduction

*Equisetum* is the sole surviving genus of the once-diverse Class Equisetopsida (Sphenopsida), a group with its origins in the Devonian and which includes such extinct arborescent members as *Calamites* of the Carboniferous. Mycorrhizal associations have been considered necessary pre-requisites for plants to colonize terrestrial environments (Pirozynski and Malloch 1975; Malloch et al. 1980), and are recognized in the plant fossil record (Strullu-Derrien and Strullu 2007), including the early sphenopsid *Astromyelon cauloides* (Agashe and Tilak 1970). Thus, a continuing role of fungal-plant symbiosis may be expected to persist in such a relictual taxon as *Equisetum*. Nevertheless, a review of the literature reveals that extant *Equisetum* spp have seldom been found to contain endorhizal fungi and, indeed, literature on *Equisetum* roots is sparse (Bierhorst 1958; Hauke 1963). Unlike Nasim et al. (1987), who concluded that *Equisetum* hosted abundant AM fungi (but claimed to have found AM structures in above-ground and in dead plant parts), most researchers report moderate (Dhillion 1993; Koske et al. 1985) to little or no endorhizal colonization (Berch and Kendrick 1982; Malloch et al. 1980; Read et al. 2000;

Schmidt and Oberwinkler 1993; Treu et al. 1996; Zhao 2000) leading to an argument that *Equisetum* may lack such associations, and perhaps has even evolutionarily lost them (Berch and Kendrick 1982).

In general, mycorrhizal research on *Equisetum* has been based on transmitted light microscopy, sometimes concentrating on arbuscules (Berch and Kendrick 1982; Schmidt and Oberwinkler 1993; Zhao 2000), although Dhillion (1993) also used soil bait studies. We have developed a high-sensitivity fluorescence microscopy method (Kaminskyj 2008) for detecting endorhizal fungi in fixed roots (Allen et al. 2006) and in herbarium specimens (Ormsby et al. 2007) that reveals previously unattainable images.

This project is part of an ongoing survey of mycorrhizae in plants collected in the Canadian High Arctic in 2004. Given the reports discussed above, we did not anticipate finding endorhizal fungi associated with *Equisetum*. Nevertheless, they were abundant in our Axel Heiberg samples, leading us to explore endorhizal fungi in *Equisetum* growing in boreal forests, where the genus is prevalent. We also extended our study of High Arctic *Equisetum* to include herbarium specimens collected at the same and nearby sites in 1988, as well as roots collected in 2006 from a range of boreal and low Arctic sites (51 ºN - 67 ºN) in northwestern Canada.

## Materials and Methods

### Plant collections

Details on collection sites are summarized in Fig. 1 and Table 1. All specimens were growing vigorously at the time of collection, and some were forming cones. The roots sampled for this project were growing from rhizomes associated with these stems, and typically had abundant root hairs (Table 2). High Arctic *Equisetum* samples were identified in the field using Porsild (1985). The remaining samples were identified using Hauke (1963, 1974, 1978) and Cody (2000). Samples from 2004 were fixed in formalin and stored at 4 ºC (Allen et al. 2006). Plants collected in 2006 were excised at the rhizome and fixed in formalin. Replicate specimens were preserved as herbarium specimen vouchers, pressed immediately after collection. Samples from 1988 collected by Basinger and colleagues were from one site on Axel Heiberg Island and two sites on Ellesmere Island. These were preserved as herbarium specimens and curated at the W. P. Fraser Herbarium, University of Saskatchewan.

Samples collected in the summers of 1988 and 2004 from the High Arctic sites were from moist to wet, coarse mineral soils underlain by permafrost about 15 cm below the surface. Samples collected in the summer of 2006 were from the Canadian Prairies, boreal forest, and western Low Arctic, spanning the latitudes 51 ºN to 67 ºN. These were mostly mesic to dry roadsides and forests; a few were near water. *Equisetum* was common at each site sampled.

*Equisetum* has a spreading rhizomatous system that produces lateral roots from nodes, which can cover many square metres and produce many shoots (Hauke 1963). Randomly selected subsamples from each collection, comprising roots associated with rhizomes connected to several neighboring shoots, were considered as single units for quantification.

### Microscopy

Samples from formalin-fixed roots were examined from each collection. Due to the large number of root hairs on most *Equisetum* species (Table 2), which typically led to clumping, it was not

possible to obtain fully randomized subsamples in the manner of Allen et al. (2006) where root segments were suspended by stirring in a volume of water. Subsamples were divided into 1- to 1.5-cm-long root segments, stained, and examined microscopically.

Root samples were removed from herbarium specimens that had sufficient root material, and prepared as described in Ormsby et al. (2007). Herbarium root samples totaled at least 10 cm per plant, and comprised 5 - 25% of the estimated root length for that specimen.

Preparation, imaging, and quantification methods followed Ormsby et al. (2007) and Kaminskyj (2008) with minor modifications as described below. Briefly, formalin-fixed and herbarium samples were cleared by autoclaving for 20 min in 10 % KOH, washed in 70 % ethanol, then bleached in freshly prepared 8:1:1 distilled water : 28 % ammonium hydroxide : 30 % hydrogen peroxide, for 2 - 8 min at room temperature with gentle agitation, until they were a pale cream colour. Cleared roots were stained in 0.05 % acid fuchsin in 85 % lactic acid, then destained in two changes of 1:1:1 distilled water : 85 % lactic acid : glycerol. Stained roots were mounted in polyvinyl alcohol glycerol medium, which was hardened at 40 ºC. Specimens were examined using wide-field and confocal epifluorescence microscopy using conditions appropriate for rhodamine fluorescence (Allen et al. 2006; Ormsby et al. 2007; Kaminskyj 2008). Transmitted light microscopy was used to assess the presence of dark septate endophyte hyphae, which do not fluoresce under these conditions.

Quantification

Fungal colonization was assessed using the multiple quantitation microintersect method (MQM; Ormsby et al. 2007; Kaminskyj 2008), that had been modified from McGonigle et al. (1990). The MQM method permits high resolution imaging and allows for independent scoring of many interaction types. Briefly, 50 to 150 equally-spaced intersections (1 or 2 mm apart, consistent for each subsample) were inspected microscopically using 20x, 40x or 63x objectives. Intersections were scored separately for: 4 - 6μm wide aseptate hyphae characteristic of arbuscular mycorrhizae (AM); arbuscules and vesicles associated with AM hyphae; 1 - 1.5 μm wide aseptate hyphae (fine endophytes, FE); arbuscules and vesicles associated with FE hyphae; 2.0 - 4.5 μm wide septate endophyte (SE) hyphae; and dark septate endophyte (DSE) hyphae. Other fungal morphologies had not been anticipated, but new categories were created to accommodate them. DSE hyphae did not fluoresce under these staining/imaging conditions, likely due to their melanized walls. Arbuscules associated with AM and FE were rare, so are not shown on Table 2. Intersections not associated with any fungi were scored separately to provide an estimate of total percent colonization. The number of root hairs per millimeter root length was counted for representative samples from each species.

Quantitative results are reported as mean ± standard error of the mean. Data were pooled by species, and compared by one-way factorial ANOVA, followed by Fisher PLSD using Statview 1.02.

## Results

*Equisetum* roots were examined for endorhizal fungal structures, and percent colonization by different morphotypes was assessed by the MQM method. Results are reported in Table 2. The fungal morphotypes are described below and illustrated in Figs. 2 - 6.

Aseptate hyphae, 4 - 6 μm wide, consistent with AM and often bearing vesicles (Fig. 2a), were present in 5 ± 1 % of intersections. Arbuscles associated with AM hyphae (Fig. 2b) were rare.

Regularly septate hyphae, 2.5 – 4 μm wide, were of two types: darkly pigmented (DSE; Fig. 3a) or hyaline (SE; Figs. 3a, b). DSE were visible with transmitted light microscopy, and did not fluoresce following lactofuchsin staining, likely due to their melanized walls. SEs with moderate levels of brown pigment did fluoresce, so were counted as SE rather than DSE. Some SEs produced sclerotium-like hyphal aggregates (Fig. 3c). Other SEs produced hyphal coils in cortical cells of young roots (Figs. 3d).

FE hyphae, 1 – 1.5 μm wide, formed both intracellular and intercellular networks (Fig. 4, arrowheads), and occasionally bore small vesicles (Fig. 4) consistent with the report of Thippayarugs et al. (1999). Arbuscles were not found in association with FE hyphae, although this association has been found in *Ranunculus* from the 2004 Axel Heiberg collection (Allen et al. 2006). Intercellular FE hyphae were commonly interspersed with SEs.

Some *Equisetum* roots contained Hartig nets and were covered with a thin mantle of extrarhizal hyphae (Fig. 5, arrows indicate optical cross sections of mantle hyphae). The mantle hyphae were septate, 2.4 ± 0.1 μm (n = 50) wide, and occasionally had clamp connections (not shown). Roots with Hartig net hyphae and mantles were found in samples growing on a stream floodplain on Axel Heiberg Island (Fig. 5), and from dry, mid-latitude sites at Meadow Lake SK, and Golden BC. The Axel Heiberg site lacked woody plants, the expected hosts for the ectomycorrhizal fungi that commonly produce Hartig nets (Peterson et al. 2004).

Some specimens had a sparse lattice of SE hyphae (Fig. 6) associated with their vascular bundles, described in Table 2 as vascular sheath hyphae. When present, this arrangement of SE hyphae was abundant. It was found in roots from the Axel Heiberg Island site, from Dempster Highway YT, and Meadow Lake SK.

*Equisetum* roots were commonly colonized by endorhizal fungi (Table 2). Considering all samples, total fungal colonization was 29.6 ± 3.2 %, ranging amongst different samples from 0 - 97 %. Thirty percent of samples (n = 25 of 85) had colonization rates above 50 % (average 74 ± 3 %). Overall, fungal colonization of *Equisetum* roots from High Arctic tundra was statistically similar to samples collected from mesic to dry sites at lower latitudes. Total colonization was significantly higher in 2004 and 2006 than in 1988. There were no other statistical differences, nor trends with respect to latitude or root hair abundance.

## Discussion

We found that *Equisetum* roots host relatively abundant, diverse endorhizal fungi, contrary to the literature (Berch and Kendrick 1982; Malloch et al. 1980; Read et al. 2000; Read and Haselwandter 1981; Schmidt and Oberwinkler 1993; Treu et al. 1996). *Equisetum* species pervade the boreal forest understory, and are important colonizers of disturbed sites, at least in our study areas: diverse localities north of 51 ºN. *Equisetum* plants produce extensive, perennial rhizomatous systems (Hauke 1963), with potentially 80% of their biomass being subterranean. *Equisetum* species can store abundant starch below ground (Hauke 1978), and for this reason *Equisetum* was cultivated for its tubers in the 1920s in upland Bolivia and Peru (Berry 1924). Interactions between *Equisetum* roots and soil mycoflora may well provide important food sources for burrowing animals and soil invertebrates.

Some of the disparity between our results and those previously reported is due to imaging methods: we used lactofuchsin-stained roots viewed with confocal epifluorescence microscopy, which is extremely sensitive. Much of the fungal colonization was by FE hyphae, which are challenging to see with conventional stains and transmitted light (Allen et al. 2006). Another source of disparity relates to definitions. Arbuscules were uncommon in our *Equisetum* samples, although many intersections had aseptate hyphae likely to be AM and FE. Like AM, FE can produce arbuscules (Allen et al. 2006) which Gianinazzi-Pearson et al. (1981) suggested were consistent with a nutrient transfer function. From our 2004 collection on Axel Heiberg, AM arbuscules were common in *Taraxacum* and *Erigeron* roots (Allen et al. 2006; Ormsby et al. 2007) and FE arbuscules were common in *Ranunculus* roots (Allen et al. 2006). Jumpponen and Trappe (1998) suggested that DSEs had a nutrient transfer function similar to mycorrhizae, and might be important in locations like the Arctic. Using cryo-analytical scanning microscopy, Ryan et al. (2003) showed that at least in agricultural plants, intercellular hyphae had a likely role in transferring phosphorus to neighboring root cells.

*Equisetum* is commonly associated with damp or wet soils, to which Berch and Kendrick (1982) and Koske et al. (1985) partially attributed their findings of relative paucity of mycorrhizal associates. However, Cooke and Lefore (1998), who did not include *Equisetum* in their study, found that 80 of 89 species they studied, including obligate hydrophytes and emergent wetland plants collected in Connecticut wetlands, had AM structures, including arbuscules, in > 70 % of samples. The earliest known AM fossils (Taylor et al. 1995), which were from a marshy environment (Trewin 1994), had distinctive arbuscules. The Axel Heiberg Island collection site was poorly drained, but we routinely found *Equisetum* in the Western Arctic and the Prairies growing abundantly in extremely dry mineral soils, including roadsides. *Equisetum* was not found near agricultural areas with apparently similar microclimate and soils as our collection sites.

In addition to hyphal networks, SE fungi are reported to form other endorhizal morphologies. Intracellular hyphal coils resembling Fig. 3b formed when *Phialophora fortinii* was used to infect *Rhododendron* roots under axenic conditions (Currah et al. 1993). SE fungi were associated with Hartig net formation in synthesized interactions (Jumpponen and Trappe 1998), so it is possible that the Hartig nets associated with *Equisetum* roots in this report were formed by SE fungi rather than the more typical ectomycorrhizal fungi.

Some SE fungi have been proposed to confer tolerance to extreme environments (Rodriguez et al. 2004). Many of our sites qualify as potentially extreme, for example, sites overlying permafrost, as well as extremely dry gravel margins adjacent to road beds. Recently, we have isolated septate endophytes from *Equisetum* growing on oil sands tailings that may have a comparable role (Bao and Kaminskyj, *in preparation*).

The fungal colonization rate for sampling years 2004 and 2006 was higher than 1988 for all types of endorhizal structure. The 1988 samples were from herbarium specimens, but a direct comparison by Ormsby et al. (2007) between formalin fixed and herbarium specimens in the Asteraceae from the same collection site in 2004 found no significant difference in fungal preservation or abundance. As with these data for *Equisetum*, Ormsby et al. (2007) found that samples collected in 2004 had higher endorhizal abundance than comparable samples back to 1982. The 2004 and 2006 years may be unusual, although the 2004 season on Axel Heiberg Island was one of the coldest of the previous two decades. The 1988 Axel Heiberg samples were collected at approximately the same time of year and from the same site as the 2004 samples.

There is yet insufficient evidence to indicate that climate change in the higher latitudes may be influencing soil microbial ecology.

The MQM method provides data that complement, but are unlike, those obtained from molecular identification. Molecular analyses can indicate fungal species richness, but depend on DNA yield and primer specificity. In contrast, the MQM method addresses the relative importance (abundance) of different types of endorhizal interaction to the host. Future studies will correlate results from these methods.

In sum, we have shown that, despite our expectation from the literature, several types of endorhizal fungi are widespread and abundant in *Equisetum* species sampled from sites spanning 51 ºN - 82 ºN. *Equisetum* hosts endorhizal fungi of three major types. Using our MQM method, overall fungal abundance was 30 %, most of which was due to fine and septate endophytes with about 20 % abundance each, with a lesser contribution from arbuscular mycorrhizal fungi. *Equisetum* species are an important component of the boreal forest and tundra, and are primary colonizers of disturbed sites, so their endorhizal associations may play a significant role in cold-region ecosystems, and therefore be potentially useful for monitoring ecological change.

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**Figure legends**

Fig. 1. Field sites used in this study. Details in Table 1.

Fig. 2. Arbuscular mycorrhiza in an *Equisetum arvense* root, Meadow Lake SK, stained with lactofuchsin and imaged with confocal epifluorescence and transmitted light microscopy. Bars = 20 μm.
a) Wide aseptate hyphae and vesicles characteristic of arbuscular mycorrhizae, shown with transmitted light microscopy as pigmented vesicles are difficult to image with fluorescence microscopy.
b) Confocal epifluorescence image of an arbuscule.

Fig. 3. Septate hyphae associated with *Equisetum* spp. roots. Samples were stained with lactofuchsin, and imaged with confocal epifluorescence microscopy. All bars = 20 μm.
a) *Equisetum praetense* root from Meadow Lake SK, stained with lactofuchsin and presented as merged confocal-epifluorescence and transmitted-light images, which illustrates the difference in staining between hyaline hyphae (white arrowhead), which fluoresce strongly, and melanized hyphae (black arrowhead), which do not fluoresce. The melanized hyphae are joined by an H-junction (H) characteristic of dark septate endophytes.
b) SE hyphae growing close to a vascular bundle in an *E. praetense* root from Golden BC. As in this image, vascular bundles in *Equisetum* are typically consist of annular rather than helical tracheids.
c) Sclerotium-like structure in the same sample as (b).
d) Intracellular coils of SE hyphae in an *Equisetum hyemale* root collected from Beaver Creek SK.

Fig. 4. Fine endophyte (FE) hyphae in an *Equisetum arvense* root. This sample was collected from Meadow Lake SK, stained with lactofuchsin and imaged with confocal epifluorescence microscopy. The FE hyphae (arrowheads) are 1-1.5 μm wide and lack septa. One FE hypha has produced a vesicle, V. Bar = 20 μm.

Fig. 5. *Equisetum variegatum* root with a hyphal mantle and Hartig net. This sample was collected from poorly drained mineral soil on Axel Heiberg Island, stained with lactofuchsin, and imaged with confocal epifluorescence microscopy. A thin mantle of hyphae (MH) surrounding the root is shown here as hyphal cross-sections (small arrows). Fine Hartig net (HN) hyphae surround but do not penetrate the cortical cells. Bar = 20 μm.

Fig. 6. *Equisetum sylvaticum* containing an unusual endorhizal phenotype, a sheath of narrow septate endophyte (SE) hyphae surrounding the vascular bundle. This sample, from the Yukon Territory Dempster Highway site, was stained with lactofuchsin and imaged with widefield epifluorescence for greater depth of focus. Both a and b are from the same specimen. Arrows indicate hyphal septa. Bar in b = 20 μm for both.

**Table 1. Sampling sites used in this study** [a]

| Location | Latitude | Longitude | Year | *Equisetum* species | Habitat |
|---|---|---|---|---|---|
| Ellesmere Island, Lake Hazen (EI-LH) | 82º01'N | 69º10'W | 1988 | *variegatum* | near lake shore |
| Axel Heiberg Island, Fossil Forest (AHI-F) | 79º55'N | 89º02'W | 2004 | *arvense* | stream floodplain |
| Axel Heiberg Island, Fossil Forest (AHI-F) | 79º55'N | 89º02'W | 2004 | *variegatum* | stream floodplain |
| Axel Heiberg Island, Fossil Forest (AHI-F) | 79º55'N | 89º02'W | 1988 | *arvense* | stream floodplain |
| Axel Heiberg Island, Fossil Forest (AHI-F) | 79º55'N | 89º02'W | 1988 | *variegatum* | stream floodplain |
| Ellesmere Island, Okse Bay (EI-OB) | 77º07'N | 86º42'W | 1988 | *arvense* | moist bench |
| Northwest Territory, Inuvik (NT-IN) | 67º26'N | 134º53'W | 2006 | *arvense* | dry roadside |
| Yukon Territory, Dempster Hwy (YT-DH) | 66º31'N | 136º31'W | 2006 | *sylvaticum* | rocky roadside |
| Yukon Territory, Dawson City (YT-DC) | 64º04'N | 139º26'W | 2006 | *arvense* | campground |
| Yukon Territory, Dawson City (YT-DC) | 64º04'N | 139º26'W | 2006 | *hyemale* | campground |
| Yukon Territory, Dawson City (YT-DC) | 64º04'N | 139º26'W | 2006 | *scirpoides* | campground |
| Yukon Territory, Dawson City (YT-DC) | 64º04'N | 139º26'W | 2006 | *variegatum* | campground |
| Yukon Territory, Whitehorse (YT-W) | 60º39'N | 135º09'W | 2006 | *arvense* | dry roadside |
| British Columbia, Summit Lake (BC-SL) | 58º39'N | 124º39'W | 2006 | *arvense* | dry slope near lake |
| British Columbia, Summit Lake (BC-SL) | 58º39'N | 124º39'W | 2006 | *scirpoides* | damp pond margin |
| Saskatchewan, Besnard Lake (SK-BL) | 55º24'N | 106º04'W | 2006 | *praetense* | roadside |
| Saskatchewan, Meadow Lake (SK-ML) | 54º45'N | 108º31'W | 2006 | *arvense* | meadow path |
| Saskatchewan, Meadow Lake (SK-ML) | 54º45'N | 108º31'W | 2006 | *hyemale* | dry riverbank |
| Saskatchewan, Meadow Lake (SK-ML) | 54º45'N | 108º31'W | 2006 | *praetense* | meadow path |
| Saskatchewan, Meadow Lake (SK-ML) | 54º45'N | 108º31'W | 2006 | *sylvaticum* | dry roadside |
| Alberta, Elk Island (AB-EI) | 53º41'N | 112º52'W | 2006 | *arvense* | dry roadside |
| Saskatchewan, White Cap (SK-WC) | 51º42'N | 106º39'W | 2006 | *hyemale* | damp pond margin |
| Saskatchewan, Beaver Creek (SK-BC) | 51º40'N | 106º39'W | 2006 | *hyemale* | dry riverbank |
| British Columbia, Golden (BC-G) | 51º17'N | 115º57'W | 2006 | *praetense* | dry roadside |

a Also see Fig. 1.

TABLE 2. Percent abundance [a] of endorhizal fungal structures [b] at sites [c] in the Canadian Arctic and Prairies

| Latitude and Site | *Equisetum sp.* (# plants) | Year | AM vesicles | AM hyphae | FE vesicles | FE hyphae | SE hyphae | DSE hyphae | Sclerotia | Vascular sheath | Hartig net | Root hairs mm$^{-1}$ root | Total Col |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 82°N EI-LH | *variegatum* (2) | 1988 | 0±0 | 0±0 | 1±1 | 14.0±1.0 | 1.0±1.0 | 0±0 | 0±0 | 0±0 | 0±0 | | 15.0±1.0 |
| 80°N AHI-F | *arvense* (4) | 1988 | 0±0 | 0±0 | 0±0 | 9.8±8.1 | 4.2±4.2 | 0±0 | 0±0 | 0±0 | 0±0 | 15 | 10.8±9.1 |
| 80°N AHI-F | *arvense* (3) | 2004 | 0±0 | 7.0±2.6 | 1±1 | 56.7±12.7 | 61.3±19.0 | 0±0 | 13.1±5.3 | 5.0±3.2 | 0±0 | - | 81.7±13.9 |
| 80°N AHI-F | *variegatum* (2) | 1988 | 0±0 | 4.5±4.5 | 0±0 | 3.0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 33 | 7.0±4.0 |
| 80°N AHI-F | *variegatum* (3) | 2004 | 1.3±1.3 | 3.3±3.3 | 3.3±3.3 | 34.3±19.2 | 29.7±13.6 | 0±0 | 1.3±0.7 | 0±0 | 0.7±0.7 | 17 | 54.0±22.0 |
| 77°N EI-OB | *arvense* (2) | 1988 | 0±0 | 0±0 | 0±0 | 2.0±2 | 1.0±1.0 | 0±0 | 14.0±10.0 | 0±0 | 0±0 | 16 | 7.0±5.0 |
| 77°N EI-OB | *variegatum* (2) | 1988 | 0±0 | 0±0 | 1±1 | 14.0±1.0 | 1.0±1.0 | 0±0 | 0±0 | 0±0 | 0±0 | - | 15.0±1.0 |
| 67°N NT-IN | *arvense* (3) | 2006 | 0±0 | 2±1.2 | 5.3±2.4 | 64.7±5.3 | 51.0±8.0 | 0±0 | 0.7±0.7 | 0±0 | 0±0 | 44 | 73.7±7.4 |
| 64°N YT-DC | *arvense* (3) | 2006 | 0±0 | 5.7±2.7 | 0±0 | 42.7±13.7 | 21.0±7.0 | 0±0 | 4.7±4.7 | 0±0 | 0±0 | - | 57.0±13.6 |
| 64°N YT-DC | *hyemale* (11) | 2006 | 0±0 | 1.3±0.5 | 0±0 | 2.4±0.6 | 4.6±0.8 | 0±0 | 0±0 | 0±0 | 0±0 | - | 6.9±1.1 |
| 64°N YT-DC | *scirpoides* (7) | 2006 | 0±0 | 2.8±0.9 | 0±0 | 2.8±0.9 | 5.1±0.9 | 0±0 | 1.0±0.8 | 0±0 | 0±0 | - | 12.0±3.1 |
| 64°N YT-DC | *variegatum* (5) | 2006 | 0±0 | 0.8±0.4 | 0±0 | 0.8±0.2 | 3.8±1.4 | 0±0 | 4.6±1.7 | 0±0 | 0±0 | - | 7.8±2.9 |
| 64°N YT-DH | *sylvaticum* (3) | 2006 | 0±0 | 8.7±5.2 | 0±0 | 0.8±0.2 | 8.3±1.2 | 0±0 | 5.7±1.8 | 8.3±3.2 | 0±0 | - | 24.3±1.7 |
| 60°N YT-W | *arvense* (2) | 2006 | 0±0 | 1.0±1.0 | 3.0±1.0 | 73.0±11.0 | 61.0±17.0 | 0±0 | 0±0 | 0±0 | 0±0 | - | 79.0±9.0 |
| 58°N BC-SL | *arvense* (2) | 2006 | 0±0 | 0±0 | 0±0 | 73.0±9.0 | 57.0±5.0 | 0±0 | 37.0±31.0 | 0±0 | 0±0 | - | 73.0±9.0 |
| 58°N BC-SL | *scirpoides* (2) | 2006 | 0±0 | 0±0 | 18.0±0.0 | 0±0 | 39.5±6.5 | 0±0 | 5.0±1.0 | 0±0 | 0±0 | - | 61.5±4.5 |
| 55°N SK-BL | *praetense* (4) | 2006 | 0.5±0.5 | 2.0±1.2 | 0±0 | 10.0±3.6 | 10.0±2.8 | 0.5±0.5 | 0±0 | 0±0 | 0±0 | 18 | 20.0±3.4 |
| 54°N SK-ML | *arvense* (3) | 2006 | 4.3±4.3 | 17.3±5.8 | 1.0±1.0 | 43.0±4.4 | 32.3±3.9 | 0±0 | 33.0±27.2 | 0.7±0.7 | 17.3±17.3 | 12 | 54.7±6.3 |
| 54°N SK-ML | *hyemale* (2) | 2006 | 0±0 | 1.0±1.0 | 1.0±1.0 | 18.0±18.0 | 9.0±9.0 | 1.0±1.0 | 0±0 | 0±0 | 0±0 | 20 | 22.0±22.0 |
| 54°N SK-ML | *praetense* (2) | 2006 | 2.5±0.5 | 3.0±3.0 | 2.0±2.0 | 15.5±6.5 | 4.0±2.0 | 4.0±2.0 | 0±0 | 0±0 | 0±0 | 19 | 24.0±12.0 |
| 54°N SK-ML | *sylvaticum*(4) | 2006 | 1.5±1.5 | 13.2±7.3 | 14.0±6.8 | 37.2±16.9 | 15.5±5.6 | 0±0 | 30.0±15.8 | 0±0 | 0±0 | 12 | 67.0±14.7 |
| 53°N AB-EI | *arvense* (4) | 2006 | 0.8±0.5 | 5.8±4.6 | 0.8±0.5 | 9.8±7.8 | 23.5±14.5 | 0±0 | 1.2±1.2 | 0±0 | 0±0 | 8 | 30.8±16.7 |

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**Table 2, continued**

| Latitude and Site | *Equisetum sp.* (# plants) | Year | AM vesicles | AM hyphae | FE vesicles | FE hyphae | SE hyphae | DSE hyphae | Sclerotia | Vascular sheath | Hartig net | Root hairs mm$^{-1}$ root | Total Col |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 51°N SK-WC | *hyemale* (3) | 2006 | 1.7±0.9 | 11.7±3.7 | 6.0±1.2 | 17.0±3.2 | 13.7±4.1 | 0±0 | 7.3±7.3 | 0±0 | 0±0 | 27 | 35.7±6.0 |
| 51°N SK-BC | *hyemale* (3) | 2006 | 0±0 | 10.0±6.11 | 5.7±0.9 | 20.3±9.8 | 0.7±0.7 | 0.7±0.7 | 0±0 | 0±0 | 0±0 | 51 | 39.0±15.4 |
| 51°N BC-G | *praetense* (3) | 2006 | 0±0 | 5.7±3.7 | 1.7±0.9 | 32.3±9.4 | 51.0±3.5 | 0±0 | 10.3±8.9 | 0±0 | 0±0 | 14 | 64.3±5.8 |

**Summary of results by species of *Equisetum***

| *Equisetum sp.* (# plants) | AM vesicles | AM hyphae | FE vesicles | FE hyphae | SE hyphae | DSE hyphae | Sclerotia | Vascular sheath | Hartig net | Root hairs mm$^{-1}$ root | Total Col |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. arvense* (29) | 0.6±0.4 | 4.2±1.2 | 1.1±0.4 | 34.3±5.6 | 29.2±5.1 | 0±0 | 9.0±3.8 | 0.6±0.4 | 1.8±1.8 | 13.6±31.6 | 44.3±6.4 |
| *E. hyemale* (19) | 0.3±0.2 | 4.3±1.4 | 1.9±0.7 | 9.2±2.7 | 8.3±2.0 | 0.2±0.1 | 1.2±1.2 | 0±0 | 0.3±0.3 | 27.5±8.4 | 18.1±7.7 |
| *E. praetense* (9) | 0.8±0.4 | 3.4±1.4 | 1.0±0.5 | 18.7±4.8 | 22.3±7.4 | 1.1±0.7 | 3.4±3.1 | 0±0 | 0±0 | 18.5±0.5 | 35.7±7.8 |
| *E. scirpoides* (9) | 0±0 | 6.2±2.3 | 0±0 | 11.0±5.5 | 9.9±3.2 | 0±0 | 1.9±0.9 | 0±0 | 0±0 | 0±0 | 23.0±7.7 |
| *E. sylvaticum* (7) | 0.9±0.9 | 11.3±4.5 | 8.0±4.6 | 22.1±11.5 | 12.4±3.3 | 0±0 | 19.6±9.8 | 3.6±2.1 | 0±0 | 12 | 48.7±11.7 |
| *E. variegatum* (12) | 0.3±0.3 | 1.9±1.0 | 1.0±0.8 | 11.9±5.8 | 9.2±4.6 | 0±0 | 2.2±0.9 | 0±0 | 0.2±0.2 | 38.5±5.5 | 20.4±7.6 |
| **Total for all samples (85)** | 0.4±0.2 | 4.6±0.7 | 1.7±0.5 | 20.4±2.7 | 17.5±2.3 | 0.2±0.1 | 5.8±1.6 | 0.5±0.2 | 0.6±0.6 | 18.8±3.4 | 29.6±3.2 |

a Determined by the multiple quantitation method (Ormsby et al. *in press*)

b AM, arbuscular mycorrhiza; FE, fine endophyte; SE, septate endophyte; DSE, dark septate endophyte; sclerotia, multicellular endorhizal structures; vascular sheath, see Fig. 6; Hartig net, Hartig net hyphae and associated extraradical mantle; Total Col, total colonization, the percentage of intersections with at least one type of fungal structure.

c Details in Table 1. Islands, Provinces, Territories: AB, Alberta; AHI, Axel Heiberg Island; BC, British Columbia; EI, Ellesmere Island; NT, Northwest Territories; SK, Saskatchewan; YT, Yukon Territory. Sites: BC, Beaver Creek (SK); DC, Dawson City (YT); DH, Dempster Highway (YT); EI, Elk Island (AB); F, Fossil Forest (AHI); G, Golden (BC); IN, Inuvik (NT); LH, Lake Hazen (EI); ML, Meadow Lake (SK); OB, Okse Bay (EI); SL, Summit Lake (BC); W, Whitehorse (YT); WC, Whitecap (SK).

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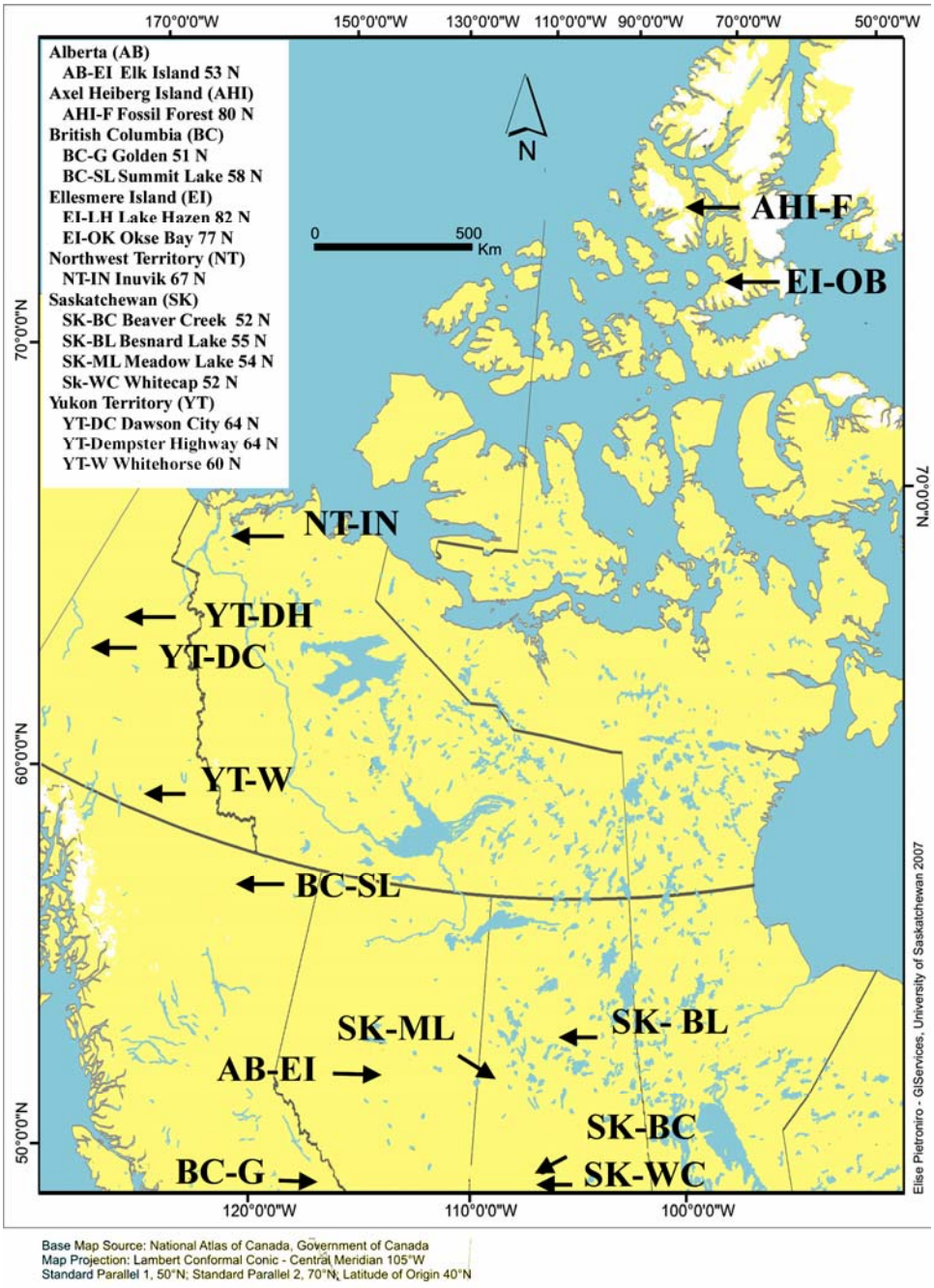



398
399 Figure 1

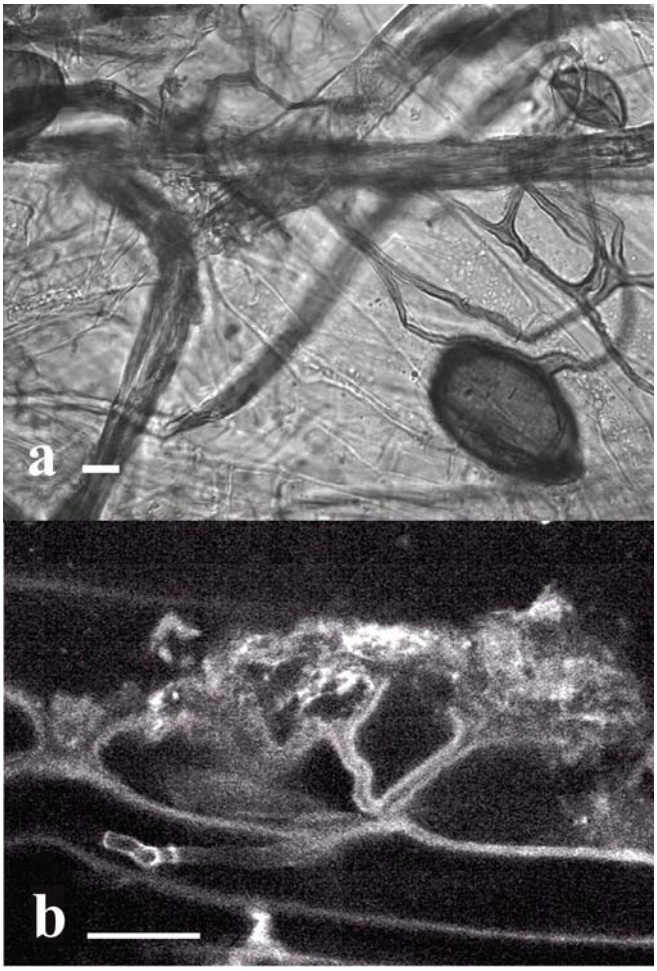
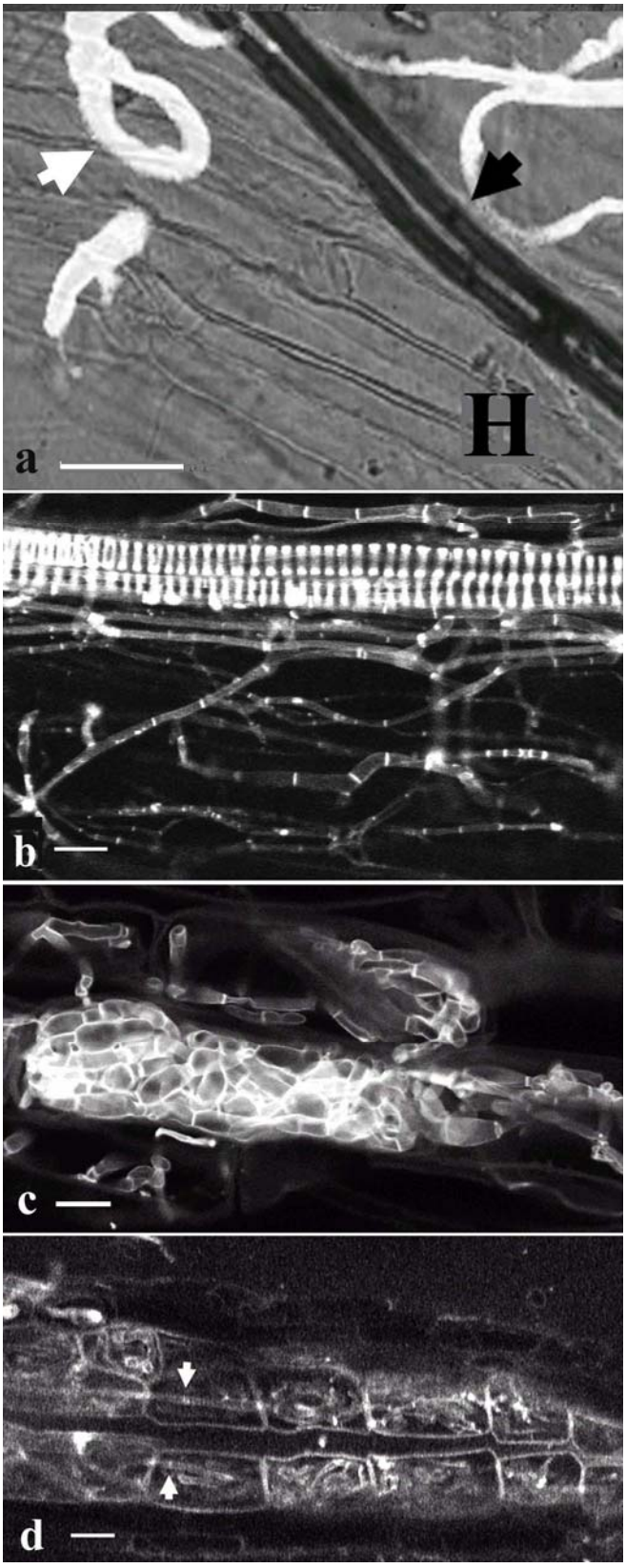



Figure 2

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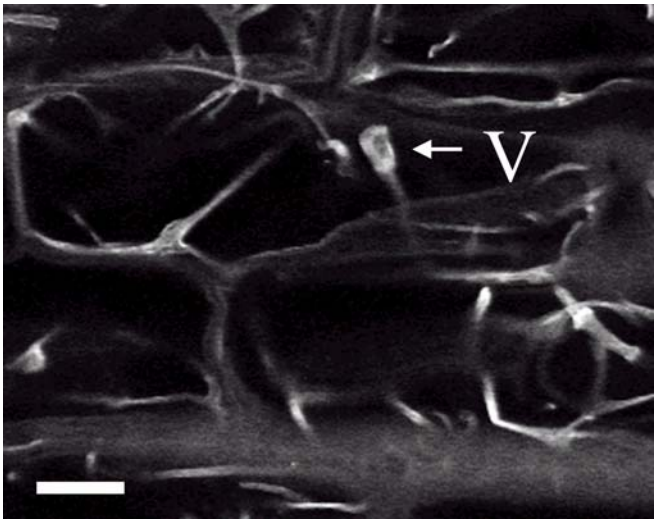


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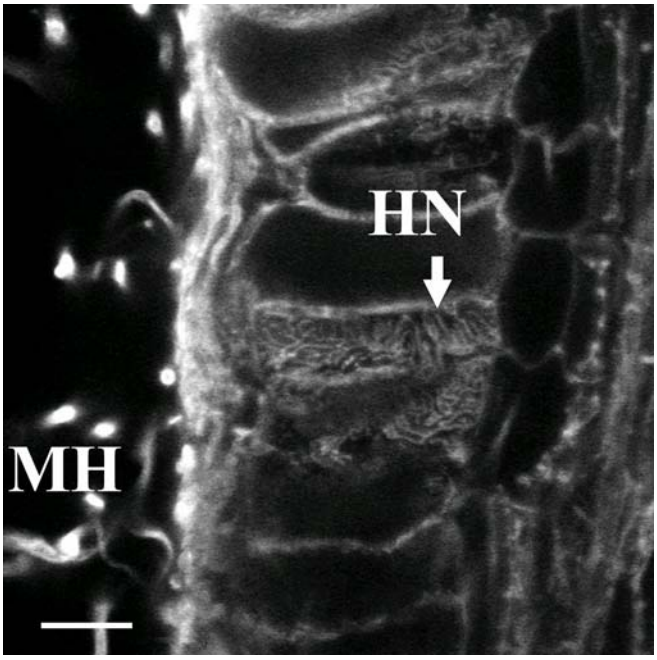


410
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412 Figure 5
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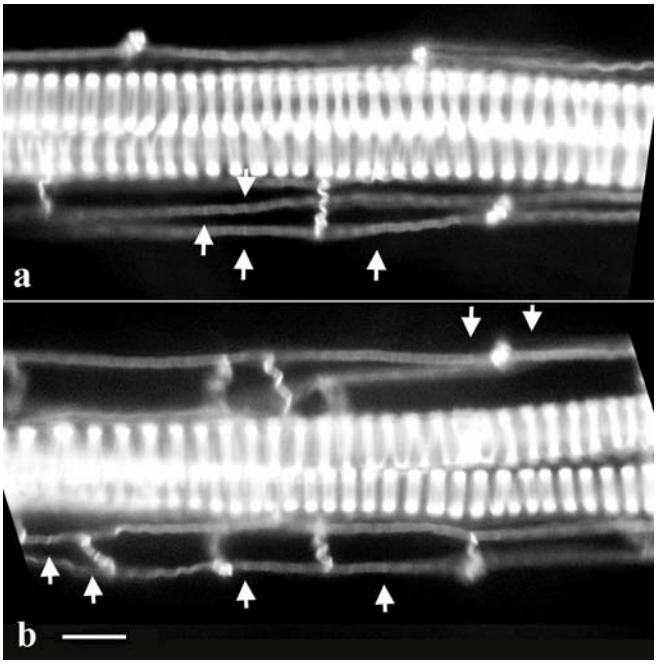


Figure 6