

Fungal endorhizal associates of *Equisetum* species from Western and Arctic Canada.Emily Hodson ^a, Fakhra Shahid ^a, James Basinger ^b, Susan Kaminskyj ^{ac}^a Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK S7N 5E2, Canada^b Department of Geological Sciences, University of Saskatchewan, 114 Science Place, Saskatoon, SK S7N 5E2, Canada^c Author for Correspondence. Telephone 1 306 966 4422, FAX 1 306 966 4461, email Susan.Kaminskyj@usask.caDOI: 10.1007/s11557-008-0574-0 The original publication is available at www.springerlink.com**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 °N), Axel Heiberg Island (80 °N), and from sites in Yukon Territory and the Prairie Provinces (67 °N - 51 °N). 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 $30 \pm 3\%$, range 0 - 97 %. Colonization rates for wide aseptate hyphae characteristic of arbuscular mycorrhizae ($5 \pm 1\%$) was significantly less than for fine endophytes ($20 \pm 3\%$) or septate endophytes ($17 \pm 2\%$). *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;

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

50 In general, mycorrhizal research on *Equisetum* has been based on transmitted light
51 microscopy, sometimes concentrating on arbuscules (Berch and Kendrick 1982; Schmidt and
52 Oberwinkler 1993; Zhao 2000), although Dhillion (1993) also used soil bait studies. We have
53 developed a high-sensitivity fluorescence microscopy method (Kaminskyj 2008) for detecting
54 endorhizal fungi in fixed roots (Allen et al. 2006) and in herbarium specimens (Ormsby et al.
55 2007) that reveals previously unattainable images.

56 This project is part of an ongoing survey of mycorrhizae in plants collected in the
57 Canadian High Arctic in 2004. Given the reports discussed above, we did not anticipate finding
58 endorhizal fungi associated with *Equisetum*. Nevertheless, they were abundant in our Axel
59 Heiberg samples, leading us to explore endorhizal fungi in *Equisetum* growing in boreal forests,
60 where the genus is prevalent. We also extended our study of High Arctic *Equisetum* to include
61 herbarium specimens collected at the same and nearby sites in 1988, as well as roots collected in
62 2006 from a range of boreal and low Arctic sites (51 °N - 67 °N) in northwestern Canada.

63

64 **Materials and Methods**

65

66 **Plant collections**

67

68 Details on collection sites are summarized in Fig. 1 and Table 1. All specimens were growing
69 vigorously at the time of collection, and some were forming cones. The roots sampled for this
70 project were growing from rhizomes associated with these stems, and typically had abundant
71 root hairs (Table 2). High Arctic *Equisetum* samples were identified in the field using Porsild
72 (1985). The remaining samples were identified using Hauke (1963, 1974, 1978) and Cody
73 (2000). Samples from 2004 were fixed in formalin and stored at 4 °C (Allen et al. 2006). Plants
74 collected in 2006 were excised at the rhizome and fixed in formalin. Replicate specimens were
75 preserved as herbarium specimen vouchers, pressed immediately after collection. Samples from
76 1988 collected by Basinger and colleagues were from one site on Axel Heiberg Island and two
77 sites on Ellesmere Island. These were preserved as herbarium specimens and curated at the W. P.
78 Fraser Herbarium, University of Saskatchewan.

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

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

88

89 **Microscopy**

90

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

93 possible to obtain fully randomized subsamples in the manner of Allen et al. (2006) where root
94 segments were suspended by stirring in a volume of water. Subsamples were divided into 1- to
95 1.5-cm-long root segments, stained, and examined microscopically.

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

99 Preparation, imaging, and quantification methods followed Ormsby et al. (2007) and
100 Kaminskyj (2008) with minor modifications as described below. Briefly, formalin-fixed and
101 herbarium samples were cleared by autoclaving for 20 min in 10 % KOH, washed in 70 %
102 ethanol, then bleached in freshly prepared 8:1:1 distilled water : 28 % ammonium hydroxide :
103 30 % hydrogen peroxide, for 2 - 8 min at room temperature with gentle agitation, until they were
104 a pale cream colour. Cleared roots were stained in 0.05 % acid fuchsin in 85 % lactic acid, then
105 destained in two changes of 1:1:1 distilled water : 85 % lactic acid : glycerol. Stained roots were
106 mounted in polyvinyl alcohol glycerol medium, which was hardened at 40 °C. Specimens were
107 examined using wide-field and confocal epifluorescence microscopy using conditions
108 appropriate for rhodamine fluorescence (Allen et al. 2006; Ormsby et al. 2007; Kaminskyj 2008).
109 Transmitted light microscopy was used to assess the presence of dark septate endophyte hyphae,
110 which do not fluoresce under these conditions.

111

112 Quantification

113

114 Fungal colonization was assessed using the multiple quantitation microintersect method (MQM;
115 Ormsby et al. 2007; Kaminskyj 2008), that had been modified from McGonigle et al. (1990).
116 The MQM method permits high resolution imaging and allows for independent scoring of many
117 interaction types. Briefly, 50 to 150 equally-spaced intersections (1 or 2 mm apart, consistent for
118 each subsample) were inspected microscopically using 20x, 40x or 63x objectives. Intersections
119 were scored separately for: 4 - 6µm wide aseptate hyphae characteristic of arbuscular
120 mycorrhizae (AM); arbuscules and vesicles associated with AM hyphae; 1 - 1.5 µm wide
121 aseptate hyphae (fine endophytes, FE); arbuscules and vesicles associated with FE hyphae; 2.0 -
122 4.5 µm wide septate endophyte (SE) hyphae; and dark septate endophyte (DSE) hyphae. Other
123 fungal morphologies had not been anticipated, but new categories were created to accommodate
124 them. DSE hyphae did not fluoresce under these staining/imaging conditions, likely due to their
125 melanized walls. Arbuscules associated with AM and FE were rare, so are not shown on Table 2.
126 Intersections not associated with any fungi were scored separately to provide an estimate of total
127 percent colonization. The number of root hairs per millimeter root length was counted for
128 representative samples from each species.

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

132

133 Results

134

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

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

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

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

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

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

163 *Equisetum* roots were commonly colonized by endorhizal fungi (Table 2). Considering all
164 samples, total fungal colonization was 29.6 ± 3.2 %, ranging amongst different samples from
165 0 - 97 %. Thirty percent of samples ($n = 25$ of 85) had colonization rates above 50 % (average
166 74 ± 3 %). Overall, fungal colonization of *Equisetum* roots from High Arctic tundra was
167 statistically similar to samples collected from mesic to dry sites at lower latitudes. Total
168 colonization was significantly higher in 2004 and 2006 than in 1988. There were no other
169 statistical differences, nor trends with respect to latitude or root hair abundance.

170

171 Discussion

172

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

183 Some of the disparity between our results and those previously reported is due to imaging
184 methods: we used lactofuchsin-stained roots viewed with confocal epifluorescence microscopy,
185 which is extremely sensitive. Much of the fungal colonization was by FE hyphae, which are
186 challenging to see with conventional stains and transmitted light (Allen et al. 2006). Another
187 source of disparity relates to definitions. Arbuscules were uncommon in our *Equisetum* samples,
188 although many intersections had aseptate hyphae likely to be AM and FE. Like AM, FE can
189 produce arbuscules (Allen et al. 2006) which Gianinazzi-Pearson et al. (1981) suggested were
190 consistent with a nutrient transfer function. From our 2004 collection on Axel Heiberg, AM
191 arbuscules were common in *Taraxacum* and *Erigeron* roots (Allen et al. 2006; Ormsby et al.
192 2007) and FE arbuscules were common in *Ranunculus* roots (Allen et al. 2006). Jumpponen and
193 Trappe (1998) suggested that DSEs had a nutrient transfer function similar to mycorrhizae, and
194 might be important in locations like the Arctic. Using cryo-analytical scanning microscopy, Ryan
195 et al. (2003) showed that at least in agricultural plants, intercellular hyphae had a likely role in
196 transferring phosphorus to neighboring root cells.

197 *Equisetum* is commonly associated with damp or wet soils, to which Berch and Kendrick
198 (1982) and Koske et al. (1985) partially attributed their findings of relative paucity of
199 mycorrhizal associates. However, Cooke and Lefore (1998), who did not include *Equisetum* in
200 their study, found that 80 of 89 species they studied, including obligate hydrophytes and
201 emergent wetland plants collected in Connecticut wetlands, had AM structures, including
202 arbuscules, in > 70 % of samples. The earliest known AM fossils (Taylor et al. 1995), which
203 were from a marshy environment (Trewin 1994), had distinctive arbuscules. The Axel Heiberg
204 Island collection site was poorly drained, but we routinely found *Equisetum* in the Western
205 Arctic and the Prairies growing abundantly in extremely dry mineral soils, including roadsides.
206 *Equisetum* was not found near agricultural areas with apparently similar microclimate and soils
207 as our collection sites.

208 In addition to hyphal networks, SE fungi are reported to form other endorhizal
209 morphologies. Intracellular hyphal coils resembling Fig. 3b formed when *Phialophora fortinii*
210 was used to infect *Rhododendron* roots under axenic conditions (Currah et al. 1993). SE fungi
211 were associated with Hartig net formation in synthesized interactions (Jumpponen and Trappe
212 1998), so it is possible that the Hartig nets associated with *Equisetum* roots in this report were
213 formed by SE fungi rather than the more typical ectomycorrhizal fungi.

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

219 The fungal colonization rate for sampling years 2004 and 2006 was higher than 1988 for
220 all types of endorhizal structure. The 1988 samples were from herbarium specimens, but a direct
221 comparison by Ormsby et al. (2007) between formalin fixed and herbarium specimens in the
222 Asteraceae from the same collection site in 2004 found no significant difference in fungal
223 preservation or abundance. As with these data for *Equisetum*, Ormsby et al. (2007) found that
224 samples collected in 2004 had higher endorhizal abundance than comparable samples back to
225 1982. The 2004 and 2006 years may be unusual, although the 2004 season on Axel Heiberg
226 Island was one of the coldest of the previous two decades. The 1988 Axel Heiberg samples were
227 collected at approximately the same time of year and from the same site as the 2004 samples.

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

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

235 In sum, we have shown that, despite our expectation from the literature, several types of
236 endorhizal fungi are widespread and abundant in *Equisetum* species sampled from sites spanning
237 51 °N - 82 °N. *Equisetum* hosts endorhizal fungi of three major types. Using our MQM method,
238 overall fungal abundance was 30 %, most of which was due to fine and septate endophytes with
239 about 20 % abundance each, with a lesser contribution from arbuscular mycorrhizal fungi.
240 *Equisetum* species are an important component of the boreal forest and tundra, and are primary
241 colonizers of disturbed sites, so their endorhizal associations may play a significant role in
242 cold-region ecosystems, and therefore be potentially useful for monitoring ecological change.

243
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330 **Figure legends**

- 331 Fig. 1. Field sites used in this study. Details in Table 1.
- 332 Fig. 2. Arbuscular mycorrhiza in an *Equisetum arvense* root, Meadow Lake SK, stained with
333 lactofuchsin and imaged with confocal epifluorescence and transmitted light microscopy.
334 Bars = 20 μ m.
- 335 a) Wide aseptate hyphae and vesicles characteristic of arbuscular mycorrhizae, shown with
336 transmitted light microscopy as pigmented vesicles are difficult to image with fluorescence
337 microscopy.
- 338 b) Confocal epifluorescence image of an arbuscule.
- 339 Fig. 3. Septate hyphae associated with *Equisetum* spp. roots. Samples were stained with
340 lactofuchsin, and imaged with confocal epifluorescence microscopy. All bars = 20 μ m.
- 341 a) *Equisetum praetense* root from Meadow Lake SK, stained with lactofuchsin and presented
342 as merged confocal-epifluorescence and transmitted-light images, which illustrates the
343 difference in staining between hyaline hyphae (white arrowhead), which fluoresce strongly,
344 and melanized hyphae (black arrowhead), which do not fluoresce. The melanized hyphae are
345 joined by an H-junction (H) characteristic of dark septate endophytes.
- 346 b) SE hyphae growing close to a vascular bundle in an *E. praetense* root from Golden BC.
347 As in this image, vascular bundles in *Equisetum* are typically consist of annular rather than
348 helical tracheids.
- 349 c) Sclerotium-like structure in the same sample as (b).
- 350 d) Intracellular coils of SE hyphae in an *Equisetum hyemale* root collected from Beaver
351 Creek SK.
- 352 Fig. 4. Fine endophyte (FE) hyphae in an *Equisetum arvense* root. This sample was collected
353 from Meadow Lake SK, stained with lactofuchsin and imaged with confocal epifluorescence
354 microscopy. The FE hyphae (arrowheads) are 1-1.5 μ m wide and lack septa. One FE hypha
355 has produced a vesicle, V. Bar = 20 μ m.
- 356 Fig. 5. *Equisetum variegatum* root with a hyphal mantle and Hartig net. This sample was
357 collected from poorly drained mineral soil on Axel Heiberg Island, stained with lactofuchsin,
358 and imaged with confocal epifluorescence microscopy. A thin mantle of hyphae (MH)
359 surrounding the root is shown here as hyphal cross-sections (small arrows). Fine Hartig net
360 (HN) hyphae surround but do not penetrate the cortical cells. Bar = 20 μ m.
- 361 Fig. 6. *Equisetum sylvaticum* containing an unusual endorhizal phenotype, a sheath of narrow
362 septate endophyte (SE) hyphae surrounding the vascular bundle. This sample, from the
363 Yukon Territory Dempster Highway site, was stained with lactofuchsin and imaged with
364 widefield epifluorescence for greater depth of focus. Both a and b are from the same
365 specimen. Arrows indicate hyphal septa. Bar in b = 20 μ m for both.

366 **Table 1. Sampling sites used in this study** ^a

367	Location	Latitude	Longitude	Year	<i>Equisetum</i> species	Habitat
368						
369	Ellesmere Island, Lake Hazen (EI-LH)	82°01'N	69°10'W	1988	<i>variegatum</i>	near lake shore
370	Axel Heiberg Island, Fossil Forest (AHI-F)	79°55'N	89°02'W	2004	<i>arvense</i>	stream floodplain
371	Axel Heiberg Island, Fossil Forest (AHI-F)	79°55'N	89°02'W	2004	<i>variegatum</i>	stream floodplain
372	Axel Heiberg Island, Fossil Forest (AHI-F)	79°55'N	89°02'W	1988	<i>arvense</i>	stream floodplain
373	Axel Heiberg Island, Fossil Forest (AHI-F)	79°55'N	89°02'W	1988	<i>variegatum</i>	stream floodplain
374	Ellesmere Island, Okse Bay (EI-OB)	77°07'N	86°42'W	1988	<i>arvense</i>	moist bench
375	Northwest Territory, Inuvik (NT-IN)	67°26'N	134°53'W	2006	<i>arvense</i>	dry roadside
376	Yukon Territory, Dempster Hwy (YT-DH)	66°31'N	136°31'W	2006	<i>sylvaticum</i>	rocky roadside
377	Yukon Territory, Dawson City (YT-DC)	64°04'N	139°26'W	2006	<i>arvense</i>	campground
378	Yukon Territory, Dawson City (YT-DC)	64°04'N	139°26'W	2006	<i>hyemale</i>	campground
379	Yukon Territory, Dawson City (YT-DC)	64°04'N	139°26'W	2006	<i>scirpoides</i>	campground
380	Yukon Territory, Dawson City (YT-DC)	64°04'N	139°26'W	2006	<i>variegatum</i>	campground
381	Yukon Territory, Whitehorse (YT-W)	60°39'N	135°09'W	2006	<i>arvense</i>	dry roadside
382	British Columbia, Summit Lake (BC-SL)	58°39'N	124°39'W	2006	<i>arvense</i>	dry slope near lake
383	British Columbia, Summit Lake (BC-SL)	58°39'N	124°39'W	2006	<i>scirpoides</i>	damp pond margin
384	Saskatchewan, Besnard Lake (SK-BL)	55°24'N	106°04'W	2006	<i>praetense</i>	roadside
385	Saskatchewan, Meadow Lake (SK-ML)	54°45'N	108°31'W	2006	<i>arvense</i>	meadow path
386	Saskatchewan, Meadow Lake (SK-ML)	54°45'N	108°31'W	2006	<i>hyemale</i>	dry riverbank
387	Saskatchewan, Meadow Lake (SK-ML)	54°45'N	108°31'W	2006	<i>praetense</i>	meadow path
388	Saskatchewan, Meadow Lake (SK-ML)	54°45'N	108°31'W	2006	<i>sylvaticum</i>	dry roadside
389	Alberta, Elk Island (AB-EI)	53°41'N	112°52'W	2006	<i>arvense</i>	dry roadside
390	Saskatchewan, White Cap (SK-WC)	51°42'N	106°39'W	2006	<i>hyemale</i>	damp pond margin
391	Saskatchewan, Beaver Creek (SK-BC)	51°40'N	106°39'W	2006	<i>hyemale</i>	dry riverbank
392	British Columbia, Golden (BC-G)	51°17'N	115°57'W	2006	<i>praetense</i>	dry roadside

393 ^a Also see Fig. 1.

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Hodson, Shahid, Basinger, Kaminskyj

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

Latitude and Site	<i>Equisetum</i> sp. (# plants)	Year	AM vesicles	AM hyphae	FE vesicles	FE hyphae	SE hyphae	DSE hyphae	Sclerotia	Vascular sheath	Hartig net	Root hairs mm ³ root	Total Col
82°N EI-LH	<i>variegatum</i> (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	<i>arvense</i> (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	<i>arvense</i> (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	<i>variegatum</i> (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	<i>variegatum</i> (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	<i>arvense</i> (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	<i>variegatum</i> (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	<i>arvense</i> (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	<i>arvense</i> (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	<i>hyemale</i> (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	<i>scirpoides</i> (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	<i>variegatum</i> (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	<i>sylvaticum</i> (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	<i>arvense</i> (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	<i>arvense</i> (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	<i>scirpoides</i> (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	<i>pratense</i> (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	<i>arvense</i> (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	<i>hyemale</i> (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	<i>pratense</i> (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	<i>sylvaticum</i> (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	<i>arvense</i> (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

Table 2, continued

Latitude and Site	<i>Equisetum</i> sp. (# plants)	Year	AM vesicles	AM hyphae	FE vesicles	FE hyphae	SE hyphae	DSE hyphae	Sclerotia	Vascular sheath	Hartig net	Root hairs mm ³ root	Total Col
51°N SK-WC	<i>hyemale</i> (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	<i>hyemale</i> (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	<i>pratense</i> (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*

<i>Equisetum</i> sp. (# plants)	AM vesicles	AM hyphae	FE vesicles	FE hyphae	SE hyphae	DSE hyphae	Sclerotia	Vascular sheath	Hartig net	Root hairs mm ³ root	Total Col
<i>E. arvense</i> (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
<i>E. hyemale</i> (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
<i>E. pratense</i> (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
<i>E. scirpoides</i> (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
<i>E. sylvaticum</i> (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
<i>E. variegatum</i> (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

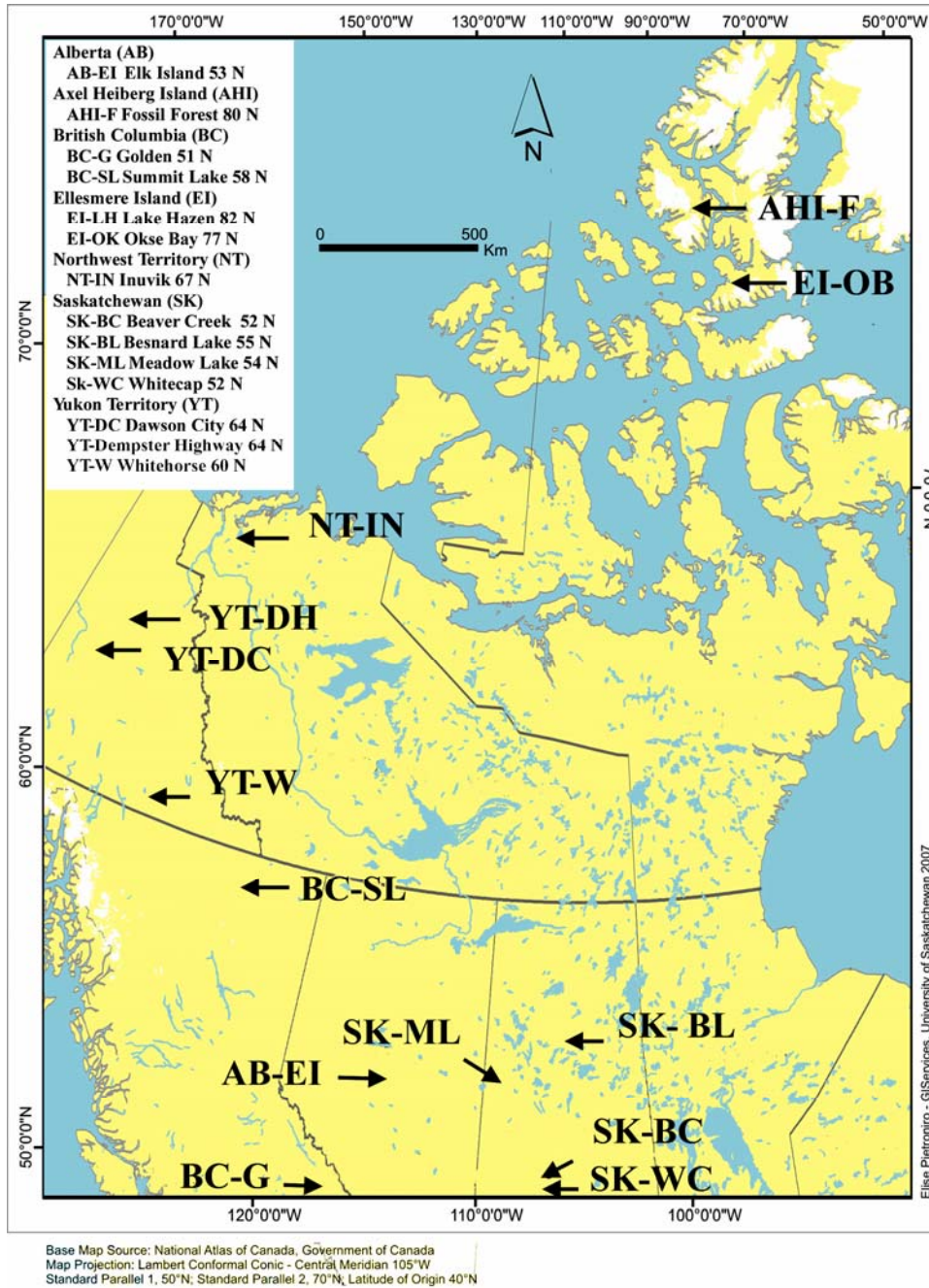
a Determined by the multiple quantitation method (Ormsby et al. *in press*)

b AM, arbuscular mycorrhiza; FE, fine endophyte; SE, septate endophyte; DSE, dark septate endophyte; sclerotia, multicellular endochizal 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.

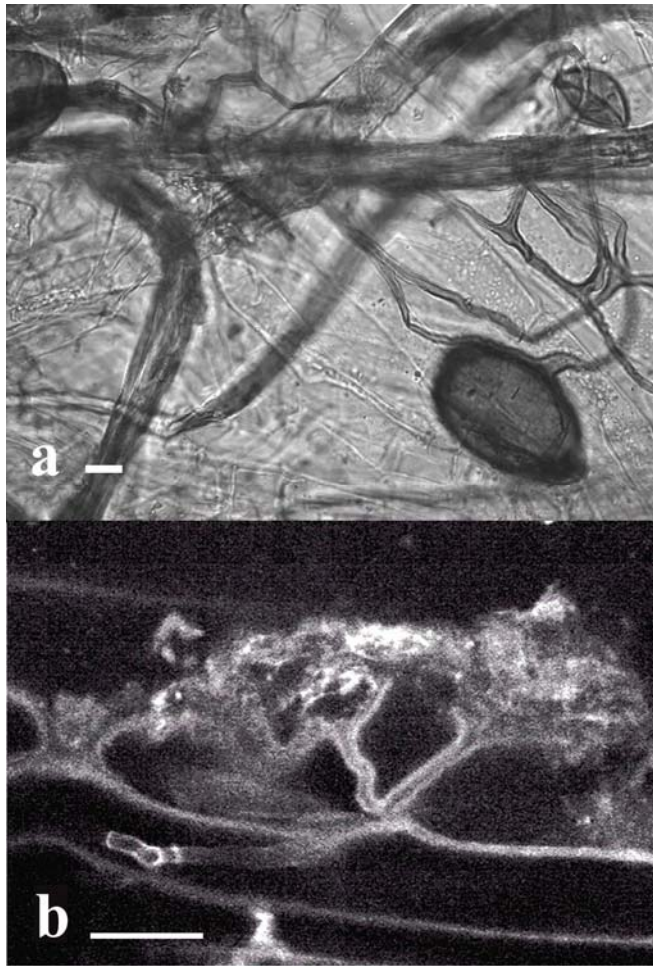
c Details in Table 1. Islands, Provinces, Territories: AB, Alberta; AH, Axel Heiberg Island; BC, British Columbia; EI, Ellesmere Island; NT, Northwest Territories; SK,

Saskatchewan; YT, Yukon Territory. Sites: BC, Beaver Creek (SK); DC, Dawson City (YT); DH, Dempster Highway (YT); EI, Elk Island (AB); F, Fossil Forest (AH); 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).



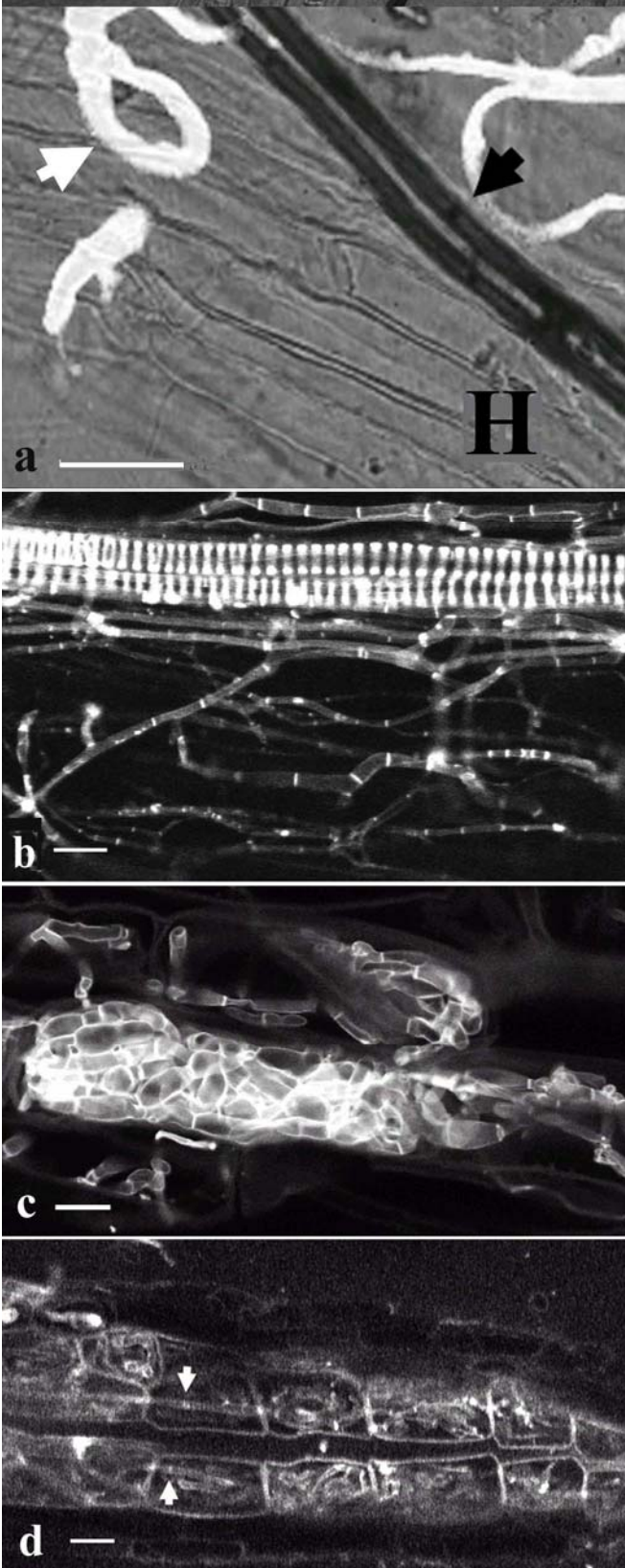
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Figure 1



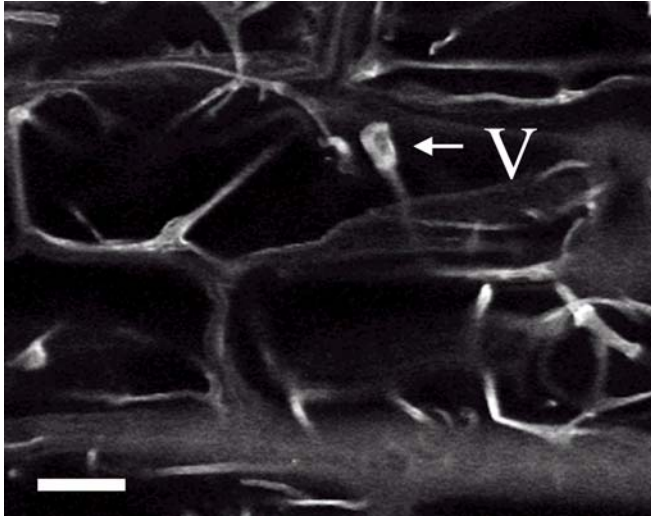
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Figure 2



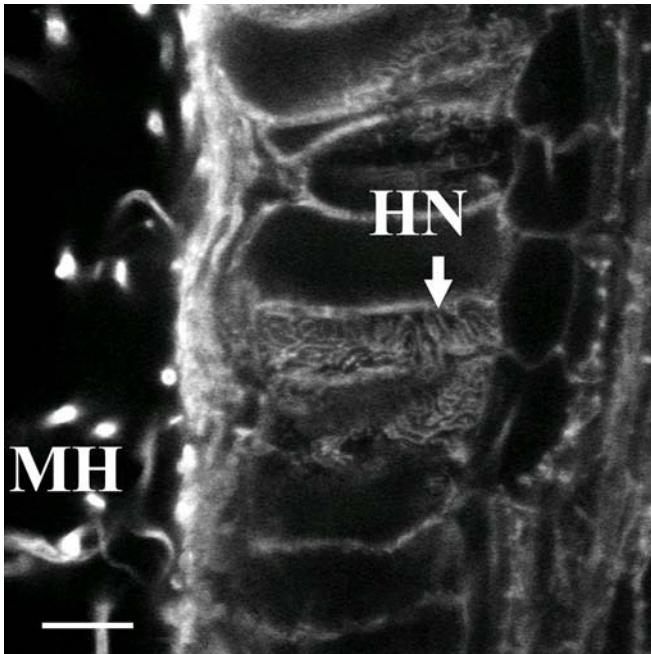
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Figure 3



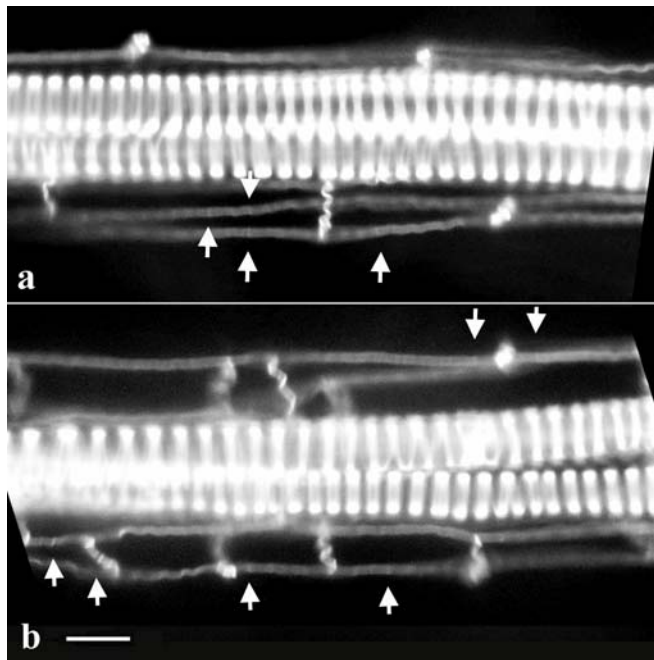
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Figure 4



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Figure 5



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Figure 6