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Root-Fungal Associations of *Colobanthus quitensis* and *Deschampsia antarctica* in the Maritime and Subantarctic

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Abstract

The two native Antarctic vascular plant species, *Colobanthus quitensis* and *Deschampsia antarctica*, were sampled from 15 points along a 1480 km latitudinal transect from South Georgia (54°S, 36°W) through to the Léonie Islands on the western Antarctic Peninsula (67°S, 68°W). Roots of plants were cleared and stained and fungal structures recorded. The commonest type of fungal association was that formed by dark septate endophytes (DSE): 32% and 27% of the root lengths of *C. quitensis* and *D. antarctica* were colonized by hyphae of these fungi, respectively. Hyaline and stained septate hyphae were also common in roots. Coarse and fine arbuscular mycorrhiza (AM) occurred in the roots of both plant species from South Georgia, and fine AM colonization with occasional arbuscules was also sporadically recorded in roots from the South Shetland Islands, suggesting functional associations between higher plants and AM symbionts. Fungal abundances were not associated with soil chemistry, but AM abundance was associated with seasonal surface air temperature, with lower colonization in more southerly, colder habitats. We conclude that DSE are widespread, and that AM fungi are sparse but present and decline in abundance at higher latitudes, in the roots of *C. quitensis* and *D. antarctica*.

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Introduction

Previous studies have found that those fungi which are consistently associated with the roots of grasses and herbs at lower altitudes and latitudes, the arbuscular mycorrhizal (AM) fungi, are either absent from, or are sparse in, the roots of plants in cold-stressed habitats (e.g. Haselwandter and Read, 1980; Read and Haselwandter, 1981; Christie and Nicolson, 1983). It has been repeatedly shown that AM fungi tend to be replaced in herbaceous roots at high latitudes and altitudes by an entirely separate group of symbionts (*sensu* de Bary, 1887), the dark septate endophytes (DSE; Bledsoe et al., 1990; Kohn and Stasovski, 1990; Väre et al., 1992; Treu et al., 1996; Laursen et al., 1997). These fungi, characterized by the formation of melanized hyphae on the surface and in the cortex of roots, and by the formation in roots of groups of spherical or elliptical cells termed microsclerotia, consist of a wide range of mostly anamorphic ascomycete taxa. They have been reported from the roots of c. 600 plant species, many of which are considered to be non-mycorrhizal (Jumpponen and Trappe, 1998a).

DSE are widespread in the roots of plants inhabiting cold-stressed environments. In the nival zone of the Alps, all nine of the plant species examined by Haselwandter and Read (1980) were found to carry DSE colonization in roots. Similarly, all but 3 of the 17 plant species sampled by Stoyke and Currah (1991) were colonized by DSE in alpine habitats in the Alberta Rocky Mountains. At Spitsbergen in the Arctic, 30 of the 76 plant species examined were found to be colonized by DSE (Väre et al., 1992), and in central Alaska, DSE microsclerotia were found in the roots of 11 of 40 plant species (Treu et al., 1996). In alpine and Arctic habitats, DSE colonization is particularly common in the roots of plants in the Caryophyllaceae, Cyperaceae, Juncaceae, and Poaceae (Read and Haselwandter, 1981; Kohn and Stasovski, 1990; Blaschke, 1991; Väre et al., 1992; Treu et al., 1996; Laursen et al., 1997).

In contrast to the knowledge that has accumulated about the presence of DSE in the roots of Arctic and alpine plants, little is known about their occurrence in the roots of Antarctic plants. The studies that have been made on DSE in the Antarctic confirm their presence in the roots of plants from South Georgia, Heard, Macquarie and the Kerguelen Islands in the subantarctic, and the South Orkney, South Shetland and Cuverville Islands in the maritime Antarctic, but have not measured the abundance of DSE structures (Christie and Nicolson, 1983; Laursen et al., 1997; Strullu et al., 1999; Frenot et al., 2005). Similarly, previous studies have shown AM propagules to be apparently present in western Antarctic Peninsula soils (Cabello et al., 1994) and AM structures to be present in subantarctic plant roots (Christie and Nicolson, 1983; Smith and Newton, 1986; Strullu et al., 1999; Frenot et al., 2005), but quantitative studies of AM abundance in Antarctica are absent from the literature. In order to quantify fungal colonization in the roots of Antarctic plants, and to test the hypothesis that DSE increase, and AM fungi decrease, in abundance in roots at higher latitudes, we hence measured the extent of colonization by fungal structures in the roots of the two native Antarctic vascular plant species, *Colobanthus quitensis* (Caryophyllaceae) and *Deschampsia antarctica* (Poaceae), along a latitudinal transect through the maritime and subantarctic.

Materials and Methods

SAMPLING

The 15 sampling sites covered a 1480 km transect from subantarctic South Georgia through to the Léonie Islands on the

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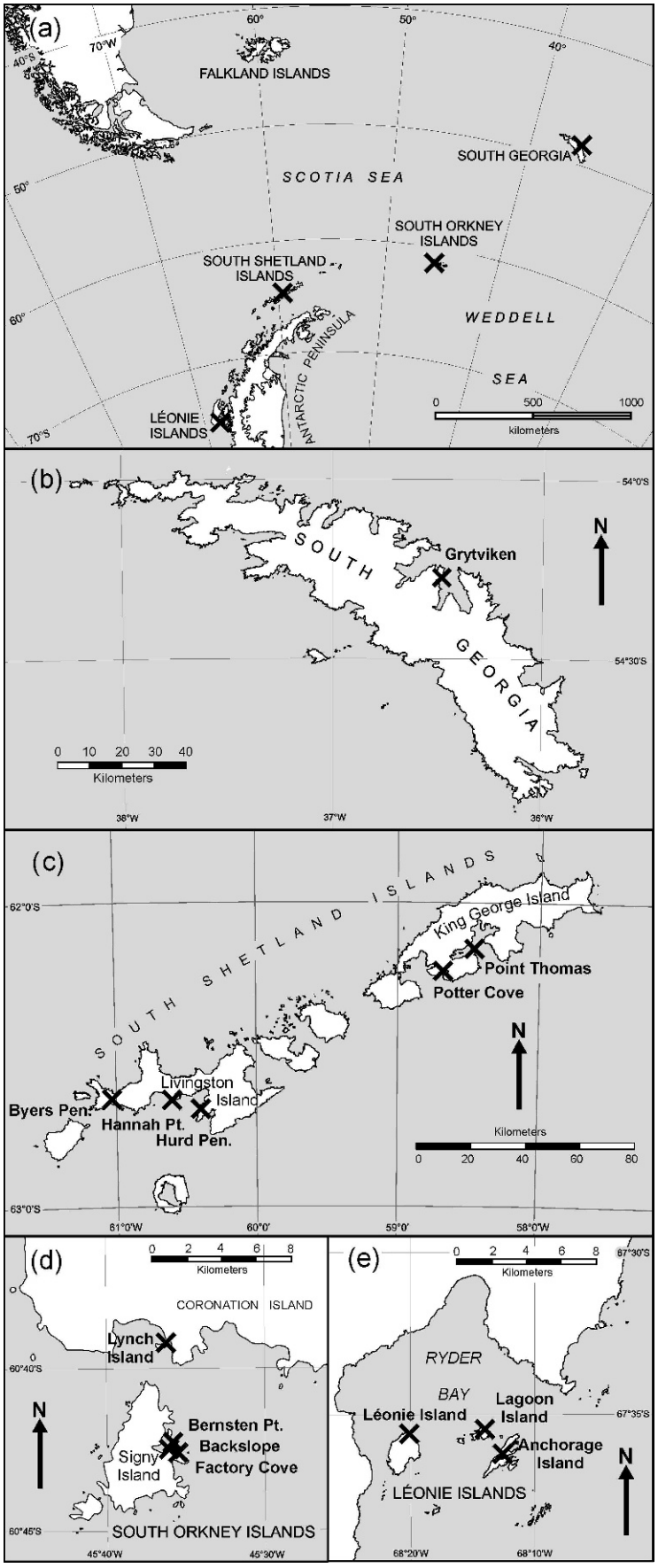

FIGURE 1. Maps showing positions of sampling sites (crosses). (a) Main map indicating locations of South Georgia, South Orkney, South Shetland, and Léonie Islands; (b)–(e) locations of sites at each of these island groups. See Table 1 for further details of sites.

TABLE 1

Locations, seasonal surface air temperatures, and soil chemistry of sampling sites.

Site	Latitude and longitude	Surface air temperature (°C)				Soil chemistry			
		spring	summer	autumn	winter	pH	Organic matter (%)	N (%)	P (%)
South Georgia									
Grytviken	54°17′S, 36°29′W	3.07 [a]	5.60 [a]	0.66 [a]	−0.57 [a]	4.72 ± 0.03	41.7 ± 8.7	1.35	0.17
South Orkney Islands		−1.11 [b]	1.15 [b]	−5.30 [b]	−8.38 [b]				
Lynch Island	60°39′S, 45°36′W	—	—	—	—	m.d.	m.d.	m.d.	m.d.
Signy Island									
Berntsen Point	60°42′S, 45°36′W	—	—	—	—	4.65 ± 0.22	40.5 ± 16.6	1.01	0.36
Backslope	60°42′S, 45°36′W	—	—	—	—	4.63 ± 0.02	60.1 ± 5.5	1.82	0.54
Factory Cove	60°42′S, 45°36′W	—	—	—	—	4.38 ± 0.05	41.1 ± 2.4	1.14	0.32
South Shetland Islands									
King George Island									
Point Thomas	62°09′S, 58°29′W	−0.11 [c]	1.40 [c]	−3.67 [c]	−4.70 [c]	4.69 ± 0.06	23.2 ± 3.6	0.99	1.33
Potter Cove	62°14′S, 58°41′W	−0.43 [d]	1.70 [d]	−2.92 [d]	−4.86 [d]	5.82 ± 0.06	3.1 ± 0.2	0.05	0.11
Livingston Island		−1.03 [e]	1.23 [e]	−3.17 [e]	−5.92 [e]				
Byers Peninsula	62°38′S, 61°04′W	—	—	—	—	5.00 ± 0.04	13.6 ± 1.4	0.68	0.83
Hannah Point	62°39′S, 60°37′W	—	—	—	—	5.00 ± 0.09	3.5 ± 0.1	0.09	0.40
Hurd Peninsula (site 1)	62°43′S, 60°25′W	—	—	—	—	5.35 ± 0.13	2.5 ± 0.1	0.12	0.38
Hurd Peninsula (site 2)	62°43′S, 60°25′W	—	—	—	—	5.57 ± 0.11	15.1 ± 3.3	0.67	0.88
Léonie Islands		−2.72 [f]	0.26 [f]	−5.75 [f]	−10.07 [f]				
Lagoon Island	67°36′S, 68°14′W	—	—	—	—	5.01 ± 0.12	45.8 ± 14.4	1.59	1.78
Léonie Island	67°36′S, 68°21′W	—	—	—	—	4.83 ± 0.37	36.7 ± 8.4	1.28	0.86
Anchorage Island (site 1)	67°36′S, 68°13′W	—	—	—	—	4.77 ± 0.04	65.8 ± 8.3	2.37	0.92
Anchorage Island (site 2)	67°36′S, 68°13′W	—	—	—	—	m.d.	m.d.	m.d.	m.d.

Air temperature data were recorded at [a]Grytviken (South Georgia), [b]Signy, [c]Henryk Arctowski and [d]King Sejong (King George Island), [e]Arturo Prat (Greenwich Island), and [f]Rothera (Adelaide Island) stations. Dashes indicate that air temperature data were unavailable for a site, but that the datum point above the dash was used as a predictor in analyses. m.d.: missing data.

western Antarctic Peninsula (Fig. 1; Table 1). Either four or five plants of *D. antarctica* were collected from each of the sites shown in Table 1, except that on Lynch Island in the South Orkney Islands. Four or five plants of *C. quitensis* were collected from each of seven sites, viz. Grytviken, Lynch Island, Point Thomas, and Potter Cove on King George Island, Byers and Hurd Peninsulas on Livingston Island, and Léonie Island (Table 1). Sites were defined as 5 m^2 areas at each of the locations. Plants with intact roots were sampled in 2003 from the South Shetland Islands on 8–11 January, the Léonie Islands on 5 February–8 March, the South Orkney Islands on 3–4 April, and from South Georgia on 7 April. Plants were placed separately in polythene bags either at 4 °C for several days or at −20 °C for several weeks prior to the microscopy analyses described below. Root zone soil was also collected from all sites, except those at Lynch Island and Anchorage Island (site 2), and stored at −20 °C for several weeks prior to the soil analyses, also described below.

MICROSCOPY ANALYSES

Where possible c. 600 mm of root was randomly selected from each plant and was washed in water and stained with aniline blue using the cold-staining method of Grace and Stribley (1991). Staining with Sudan IV (Barrow and Aaltonen, 2001) was attempted but results were variable and hence this method was not used further.

Stained root fragments were mounted on glass slides (c. 200 mm per slide) and were scored at ×400 magnification for the percentage of root length colonized by fungal structures using the magnified intersections method (McGonigle et al., 1990). A magnification of ×1000 was used in cases where more precise examination was needed. Counts were made along seven equidistantly spaced transects across each slide, leading to a mean of 178 intersections per plant. The minimum and maximum number of intersections per plant were 48 and 343, respectively. As in Ruotsalainen et al. (2002), the crosshairs of the eyepiece were aligned perpendicularly to the root vascular cylinder when scoring, rather than to the point at which a root first met the crosshairs.

Intraradical fungal structures were recorded in five categories, as follow: (1) DSE hyphae (2–6 μm diameter) with frequent septa and melanized walls; (2) DSE microsclerotia, consisting of clusters of melanized or aniline blue-staining circular or elliptical cells (10–12 μm diameter), typically arising from DSE hyphae; (3) hyaline septate hyphae (1–6 μm diameter), not staining with aniline blue; (4) stained septate hyphae (2–6 μm diameter), staining with aniline blue; and (5) arbuscular mycorrhiza, either with coarse, aseptate hyphae (5–10 μm diameter) and branched arbuscules with or without the presence of intracellular vesicles, or fine endophyte, with thin (1–3 μm diameter), aniline blue-staining hyphae, with or without the presence of vesicles and arbuscules. Coarse and fine AM structures could not always be reliably differentiated and were hence scored in a single category. The frequencies of the five types

of fungal structure were also calculated for each site and plant species as no. plants colonized/no. plants sampled × 100%.

SOIL ANALYSES

Five replicate soil samples from each site were analyzed for pH and organic matter content. Soil pH was measured by adding double the volume of distilled water to each soil sample and recording pH after 1 h, or after the meter reading had stabilized, with a pH meter fitted with a glass electrode (Hanna Instruments, Leighton Buzzard, U.K.). Soil organic matter was measured by heating 1 g of sieved (<2 mm) soil at 550 °C in a muffle furnace for 2 h prior to weighing. Owing to a lack of material, the remaining soil samples from each site were pooled and analyzed for total nitrogen (N) and phosphorus (P). The samples were dried at 80 °C, passed through a 500 μm sieve, dried for 24 h at 80 °C, and digested with sulfuric acid and salicylic acid mixture with lithium sulfate and copper sulfate. Total N and P were then determined by the Kjeldahl method (Allen, 1989).

CLIMATE DATA

Seasonal air temperatures, recorded at six research stations (Table 1) over at least 14 years between 1975 and 2006, were obtained from the READER data set (http://www.antarctica.ac.uk/met/READER/). The stations were within 2 km of each of the sampling sites on South Georgia, Signy, and King George Islands, within 10 km of sites on Lynch and the Léonie Islands, and within 83 km of sites on Livingston Island, on which there are no research stations.

STATISTICAL ANALYSES

Statistical analyses of all data were performed using MINITAB (release 13.31). Two-way ANOVA (general linear model) was used to test whether or not the percentage of root length colonized by fungi differed between sites and plant species. All data were arcsine transformed prior to analysis. Of the 15 sites from which plants were collected, both plant species occurred at Grytviken, Point Thomas, and Potter Cove on King George Island, Byers and Hurd Peninsulas on Livingston Island, and Léonie Island. Only data from these sites were therefore included in these analyses.

Linear regression was used to test for associations between the percentage of root length colonized by fungal structures and latitude, seasonal surface air temperatures, and soil pH, organic matter content, and N and P concentrations. Data for each of these predictor variates were pooled for each site and the analyses were made separately for each plant species, so that d.f. for *C. quitensis* and *D. antarctica* were 6 and 13, respectively. Fungal colonization data from Grytviken, sites on the South Orkney Islands, Point Thomas, Potter Cove, sites on Livingston Island, and those on the Léonie Islands were correlated against air temperature data from Grytviken, Signy, Henryk Arctowski, King Sejong, Arturo Prat, and Rothera research stations, respectively (Table 1). Data were also ordinated by principal components analysis in order to determine the factors that best discriminated sites.

Results

DSE COLONIZATION

The surfaces of *C. quitensis* and *D. antarctica* roots were frequently colonized by loose wefts of DSE hyphae. The hyphae often penetrated cortical cells, where they formed microsclerotia. The structure of microsclerotia differed between the two plant species, with plate-like structures (up to 50 × 100 μm) or clusters of cells (up to 30 × 170 μm) forming in *C. quitensis* roots, and smaller clusters of microsclerotia (up to 30 × 80 μm) forming in those of *D. antarctica*.

DSE hyphal colonization was common in roots (Fig. 2a), with hyphae observed in the roots of plants from all sites. The mean lengths of *C. quitensis* and *D. antarctica* roots colonized by DSE hyphae were 32.2% and 27.1%, respectively (Fig 2a). The abundance of DSE microsclerotia was lower than of hyphae in the roots of both plant species (Fig. 2b); mean lengths of *C. quitensis* and *D. antarctica* roots colonized by these structures were 1.4% and 0.6%, respectively. Microsclerotia were absent from the roots of *D. antarctica* sampled from six sites (Fig. 2b). At sites where microsclerotia were recorded in roots, their mean frequencies were 55% (*C. quitensis*) and 40% (*D. antarctica*).

Site had a highly significant effect on the length of root colonized by DSE hyphae (Table 2), with wide variation in DSE hyphal colonization between sampling sites (Fig. 2a). When data were pooled for both plant species, DSE hyphae were more abundant in roots from Point Thomas than in those from Grytviken. Plant species did not affect the length of root colonized by DSE hyphae (Table 2). The interaction between plant species and site did, however, have a significant effect on DSE hyphal colonization (Table 2), apparently because of the significant difference in the abundances of DSE hyphae in *C. quitensis* and *D. antarctica* roots from site 2 on the Hurd Peninsula. Site also affected the length of root colonized by DSE microsclerotia (Table 2): these structures were more common in roots from Léonie Island than they were in those from the Hurd and Byers Peninsulas (Fig. 2b). Plant species also had a marginally significant effect on the length of root colonized by DSE microsclerotia (Table 2), with microsclerotia occurring over twice as often in the roots of *C. quitensis* than in those of *D. antarctica*. The site × plant species interaction term was not significant for DSE microsclerotia (Table 2).

Regression analyses indicated no associations between the length of root colonized by DSE hyphae or microsclerotia and latitude for either plant species ($r^2 = 0.00 - 0.34$; all $P > 0.05$). Similarly, the length of root colonized by these structures did not correlate with seasonal surface air temperatures ($r^2 = 0.00 - 0.44$; all $P > 0.05$) or soil chemical parameters ($r^2 = 0.02 - 0.12$; all $P > 0.05$).

NON-DSE COLONIZATION

Hyaline septate hyphae were commonly recorded in roots (Fig. 3a), with these hyphae present in the roots of all plants from all sites sampled. The mean lengths of *C. quitensis* and *D. antarctica* roots colonized by these hyphae were 10.9% and 18.2%, respectively (Fig. 3a). Site had a highly significant effect on hyaline septate hyphal colonization (Table 2): when data were pooled for both plant species, these hyphae were more abundant in roots from Point Thomas on King George Island than in those from Grytviken on South Georgia. Plant species also significantly affected hyaline septate hyphal colonization (Table 2): these hyphae were more abundant in the roots of *D. antarctica* than in those of *C. quitensis*. There was also a highly significant interactive effect of site and plant species on hyaline septate hyphae (Table 2), caused principally by more abundant hyaline septate hyphae in *D. antarctica* than in *C. quitensis* roots from Point Thomas.

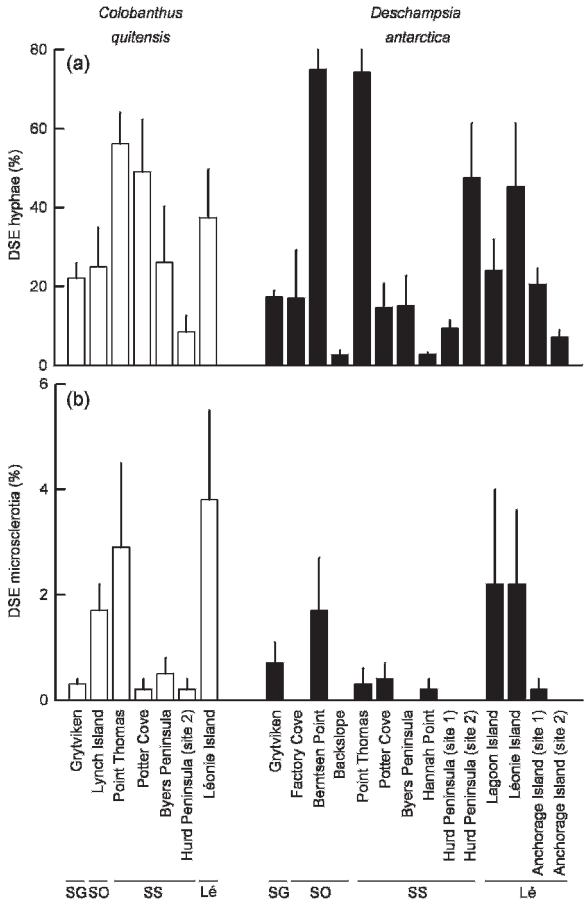

FIGURE 2. Percentage of root length of *Colobanthus quitensis* (open bars) and *Deschampsia antarctica* (closed bars) colonized by (a) DSE hyphae and (b) DSE microsclerotia. Values are means of 4–5 replicates + s.e. Abbreviations at base of Figure indicate the island groups on which the sampling sites are located (SG, South Georgia; SO, South Orkney Islands; SS, South Shetland Islands; Lé, Léonie Islands). Note that *y*-axes are not identically scaled and that *C. quitensis* was absent from eight of the sites from which *D. antarctica* was sampled.

Stained septate hyphae were also commonly recorded in roots (Fig. 3b), with these hyphae present in the roots of all plants at all sites from which they were sampled. Mean lengths of *C. quitensis* and *D. antarctica* roots colonized by stained septate hyphae were 19.2% and 12.7%, respectively (Fig. 3b). There was a highly significant main effect of site on stained septate hyphal colonization (Table 2), owing to the wide differences between sites in the abundances of these hyphae (Fig. 3b). Plant species also had a significant effect on the length of root colonized by stained septate hyphae (Table 2): these hyphae were more abundant in *C. quitensis* than in *D. antarctica* roots. There was also a highly significant interactive effect of site and plant species on the occurrence of these hyphae in roots (Table 2), principally caused by more abundant stained septate hyphae in the roots of *C. quitensis* than in those of *D. antarctica* from Grytviken.

Coarse and fine AM structures were sporadically recorded in the roots of *C. quitensis* and *D. antarctica* (data not shown). Hyphae, vesicles, and arbuscules were observed in *C. quitensis* roots from Grytviken on South Georgia (mean root length colonized [RLC] ± s.e. = 14.5 ± 3.6%, frequency = 100%) and fine endophyte lacking arbuscules was also recorded in *C. quitensis* roots from Point Thomas and Potter Cove on King George Island (mean RLC = 1.0 ± 1.0% and 0.2 ± 0.2%, respectively; both frequencies = 20%). Coarse and fine AM hyphae were also present in the roots of *D. antarctica* from Grytviken (mean RLC = 9.6 ± 4.4%, frequency = 100%) and fine endophyte was also observed in *D. antarctica* roots from Hannah Point and the Hurd Peninsula (site 2), both on Livingston Island in the South Shetland Islands (mean RLC = 0.2 ± 0.2% and 9.4 ± 4.4%, respectively; both frequencies = 25%). Occasional arbuscules were recorded in

TABLE 2

***F* ratios and *P* values from two-way ANOVAs testing the effects of site and plant species on the percentage of root length colonized by fungal structures in *Deschampsia antarctica* and *Colobanthus quitensis*. Significant *P* values are marked in bold. Degrees of freedom for site, plant species, site × plant species, and error were 5, 1, 5, and 41, respectively.**

	site		plant species		site × plant species	
	F ratio	*P* value	*F* ratio	*P* value	*F* ratio	*P* value
DSE colonization						
hyphae	5.21	**0.001**	0.25	0.619	3.74	**0.007**
microsclerotia	4.35	**0.003**	2.91	0.096	0.86	0.519
Non-DSE colonization						
hyaline septate hyphae	20.56	<**0.001**	5.53	**0.024**	7.23	<**0.001**
stained septate hyphae	6.75	<**0.001**	9.81	**0.003**	8.34	<**0.001**
arbuscular mycorrhiza	3.76	**0.007**	0.07	0.798	0.90	0.488

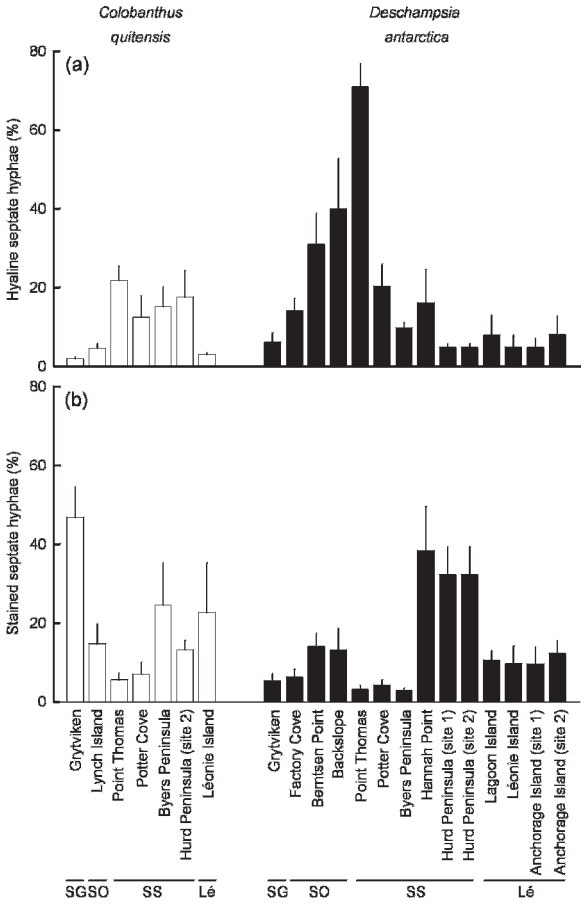

FIGURE 3. Percentage of root length of *Colobanthus quitensis* (open bars) and *Deschampsia antarctica* (closed bars) colonized by (a) hyaline septate and (b) stained septate hyphae. Values are means of 4–5 replicates + s.e. Abbreviations at base of figure as in Figure 2. Note that *C. quitensis* was absent from eight of the sites from which *D. antarctica* was sampled.

the roots of *D. antarctica* from the Hurd Peninsula. AM structures were absent from roots sampled from all other sites. There was thus a significant main effect of site on root length colonized by AM structures (Table 2), but plant species and the site × plant species interaction did not affect the abundance of AM fungi (Table 2).

Regression analyses indicated no significant associations for either plant species between the length of root colonized by hyaline or stained septate hyphae and latitude, seasonal surface air temperatures, or soil chemical parameters (r^2 = 0.00–0.56, all $P >$ 0.05). However, the length of root colonized by AM structures in *C. quitensis* roots declined with latitude (r^2 = 0.71, slope = −1.2, P = 0.018). There was a similar, but marginally significant, reduction in AM colonization in *D. antarctica* roots at higher latitudes (r^2 = 0.27, slope = −0.5, P = 0.056). There were also significant positive associations between the length of root colonized by AM fungi in both plant species and spring, summer, autumn, and winter surface air temperatures (*D. antarctica*; r^2 = 0.38–0.47, slopes = 0.7–1.8, P = 0.024–0.007; and *C. quitensis,* r^2 = 0.62–0.94, slopes = 1.4–3.0, P = 0.037–0.001), with the best fits recorded for summer air temperatures in both plant species. These associations were not significant when data for South Georgia were removed from the analyses (all $P >$ 0.18). Each of the associations, except that between AM colonization in *C. quitensis* roots and winter air temperature, were significant ($P <$ 0.05) when data for Livingston Island were removed from the analyses.

PRINCIPAL COMPONENTS ANALYSIS

The first and second principal components explained 78% of the variation in the data. The first component was dominated by stained septate hyphae, AM structures, and all mean seasonal temperatures. The second component was dominated by N, organic matter, and pH. South Georgia had a high negative loading on the first axis, clearly separating it from the other sites. All other sites were separated out on the second axis (data not shown).

Discussion

This is the first quantitative study into the occurrence of root-associated fungi in the maritime and subantarctic. It shows that DSE, the hyphae and microsclerotia of which were similar to those reported in the roots of alpine and Arctic plant species (cf. Haselwandter and Read, 1980; Read and Haselwandter, 1981; Bledsoe et al., 1990; Kohn and Stasovski, 1990; Treu et al., 1996; Laursen et al., 1997), are the most frequent fungal colonists of the roots of *Colobanthus quitensis* and *Deschampsia antarctica*. Less frequent were hyaline and stained septate hyphae, which have also previously been recorded in plant roots from alpine and Arctic habitats (e.g. Kohn and Stasovski, 1990; Stoyke and Currah, 1991; Väre et al., 1992). Lastly, arbuscular mycorrhizas were sparse, but present, in the roots of *C. quitensis* and *D. antarctica,* corroborating a previous report showing the apparent presence of AM propagules in soil on the western Antarctic Peninsula (Cabello et al., 1994). In agreement with previous studies that found few or no arbuscules in the roots of High Arctic plants (Bledsoe et al., 1990; Kohn and Stasovski, 1990), we recorded arbuscules only sporadically in roots sampled from the maritime Antarctic. They were, however, occasionally found in the roots of *D. antarctica* from Livingston Island in the South Shetland Islands, suggesting that there may be a functional association between the plant species and AM symbionts at this location. This is the most southerly record of AM structures

in roots: Christie and Nicolson (1983) and DeMars and Boerner (1995) examined *C. quitensis* and *D. antarctica* roots from seven locations south of Livingston Island in the maritime Antarctic, but found no evidence of AM colonization.

AM fungi were less common in roots from more southerly sites in the present study, confirming the hypothesis that these symbionts decrease in abundance at higher latitudes. In agreement with previous studies in cold-stressed habitats (Haselwandter and Read, 1980; Read and Haselwandter, 1981; Ruotsalainen et al. 2004), fine endophyte was the dominant form of AM recorded at higher latitudes. The reductions in AM fungi may have been owing to the harsher climate at these latitudes, since significant positive associations between root lengths colonized by AM and seasonal surface air temperatures were also recorded. These data are in broad agreement with many other studies that have shown arbuscular mycorrhizas to be infrequent in roots in cold-stressed habitats (Christie and Nicolson, 1983; Bledsoe et al., 1990; Kohn and Stasovski, 1990; Treu et al., 1996; Laursen et al., 1997). Our data should be interpreted with some caution, however, as the air temperatures for the sites on Livingston Island were recorded some 83 km away on Greenwich Island. Nevertheless, when data for these sites were removed from regression analyses, all but one of the associations between the root length colonized by AM fungi and seasonal air temperatures were significant, suggesting that AM abundance was indeed negatively associated with habitat temperature.

Associations between AM abundance and latitude or seasonal air temperatures were not significant when data for South Georgia were removed from regression analyses. Data from this subantarctic island, which experiences substantially higher air temperatures than the other maritime Antarctic sites studied here, thus had a significant influence on these analyses. This was borne out by the principal components analysis, which indicated that seasonal air temperatures, AM structures, and stained septate hyphae dominated the first component, separating South Georgia from the maritime Antarctic sites. The latter were each separated by soil chemistry. However, it was unclear from these, and from the regression analyses, as to why site had such a consistent effect on fungal abundances in roots, with the extent of colonization often varying widely between nearby sites. Wide variation in fungal abundances in roots from the same locality is a common feature of previous studies (e.g. Read and Haselwandter, 1981; Strullu et al., 1999; Frenot et al., 2005), but the causes of this wide variability between sites are unclear. In a previous study, DSE abundance in roots was associated with soil P (Ruotsalainen et al., 2002), but we found no evidence that soil P, nor N, pH, or organic matter content, explained the variation between sites in fungal colonization of roots. It is thus probable that other factors, such as the microclimate at each site or root density, neither of which we measured, may have determined the abundance of fungi in the roots of *C. quitensis* and *D. antarctica*.

Previous studies have alluded to the relationship between DSE colonization and either altitude or latitude, and, by implication, climate, suggesting that DSE become more abundant in roots in cold-stressed habitats (Haselwandter and Read, 1980; Currah and Van Dyk, 1986; Bledsoe et al., 1990; Väre et al., 1992). However, we could find no relationship between either latitude or climate and DSE colonization along the transect in the present study: for example, hyphae of these fungi were more abundant in roots from Point Thomas in the South Shetland Islands than in those from South Georgia. Ruotsalainen et al. (2004) similarly found no increase in the root length colonized by DSE hyphae and microsclerotia in roots sampled along an altitudinal gradient between 0 and 1400 m a.s.l. at Mount Paras in northern Norway. Hence, further studies are needed to test explicitly whether or not DSE structures do become more abundant in colder habitats. Latitude and climate were similarly poor predictors for the abundances of hyaline and stained septate hyphae in the current study.

We did not find a main effect of plant species on AM colonization in our study. This was surprising, given that *C. quitensis* is in the Caryophyllaceae, a family thought to be primarily non-mycorrhizal (Cripps and Eddington, 2005; Wang and Qui, 2006), whereas *D. antarctica* is in the Poaceae, a family that routinely hosts AM fungi. Nevertheless, differences were observed between the two plant species in the abundances of other root-associated fungi. Stained septate hyphae were more abundant in the roots of *C. quitensis* than in those of *D. antarctica*, whereas hyaline septate hyphae were commoner in the roots of the latter species than the former. Although differences between plant species in the extent of fungal colonization of roots are widely reported in the literature (e.g. Read and Haselwandter, 1981; Ruotsalainen et al., 2004), previous studies have not identified interspecific differences in the structures formed by DSE in roots. We found a consistent difference between *D. antarctica* and *C. quitensis* roots in the size of DSE microsclerotia, possibly indicating that the same DSE taxa in the roots of either species responds differently to the two hosts. Alternatively, although host specificity has yet to be demonstrated in DSE fungal associations (Mandyam and Jumpponen, 2005), the different microsclerotia in the roots of *D. antarctica* and *C. quitensis* might indicate the presence of different dominant DSE taxa in the two hosts.

The current study found that DSE hyphae occupied 32% and 27% of the root lengths of *C. quitensis* and *D. antarctica*, respectively. These abundances are comparable to those of DSE colonization found in plant roots in other cold-stressed habitats. For example, Mullen et al. (1998) found that 20–29% of the root length of the alpine herb *Ranunculus adoneus* in the North American Rockies was colonized by DSE, and suggested that dark septate fungi may contribute to the early-season uptake of soil N by the herb. Inoculation experiments under laboratory or glasshouse conditions have shown that plant biomass can be more than doubled when DSE occupy 10–30% of root length (Jumpponen and Trappe, 1998b; Newsham, 1999), implying that DSE might exert effects on the growth of *D. antarctica* and *C. quitensis* in the natural environment. This will be the focus for a future study.

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