

Environmental effects on grass-endophyte associations in the harsh conditions of south Patagonia

M. Victoria Novas¹, Marta Collantes² & Daniel Cabral¹

¹Lab. De Micología., Departamento de Biodiversidad y Biología Experimental (DBBE), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina; and ²Museo Argentino de Ciencias Naturales (MACN), Buenos Aires, Argentina

Correspondence: M. Victoria Novas, Lab. De Micología., Departamento de Biodiversidad y Biología Experimental (DBBE), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pab. II, 4° piso, Cdad. Universitaria. CP C 1428EHA, Buenos Aires, Argentina. Tel./fax: +54 11 47872706; e-mail: vicnovas@bg.fcen.uba.ar

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Abstract

Cool-season grasses are frequently infected by *Neotyphodium* endophytes and this association is often considered as a mutualistic symbiosis. We examined the incidence of *Neotyphodium* in populations of *Bromus setifolius*, *Phleum alpinum* and *Poa spiciformis*, native and wide-spread grasses from south Patagonia, Argentina. The incidence of 36 populations of *Bromus setifolius* was studied in association with climatic and soil variables. 31 populations of *Ph. alpinum* were sampled in five different plant communities. Seventeen populations of *P. spiciformis* were sampled in three different plant communities. The association between incidence and climatic variables in *Ph. alpinum* and between incidence and soil fertility in *P. spiciformis* was investigated. In *B. setifolius* endophyte incidence was positively correlated with annual average rainfall contrary to the results found in *Ph. alpinum*. All the populations of *P. spiciformis* were infected by endophytes and the incidence was associated with plant community. The *Neotyphodium*-grass interaction is variable in natural populations, supporting the increasing evidence that the *Neotyphodium*-host interaction depends, in many cases, on the environmental conditions. Field observations suggest that in detrimental low growth conditions the association is not favoured, leading to a decrease in the endophyte frequency of infection or even to the complete loss of the association.

Introduction

Many cool-grasses are infected by asexual endophytes of the genus *Neotyphodium* (Glenn, Hanlin and Bacon). These endophytes are obligate seed-borne fungi that form asymptomatic infections within the aerial tissues of the host plant, although they are related to, and derived from, pathogenic, sexually-reproducing clavicipitaceous fungi (*Epichloë* Tul.) (Schardl, 1996; Schardl *et al.*, 1997). Endophyte-grass associations have been associated with livestock toxicosis in animals feeding on tall fescue (*Lolium arundinaceum* = *Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) (Bacon, 1995). Plants infected by endophytes can obtain benefits such as increased tillering, reproduction and plant growth, enhanced drought tolerance and decreased susceptibility to insect feeding (Johnson *et al.*, 1985; Latch *et al.*, 1985; Siegel *et al.*, 1987; Lyons *et al.*, 1990; Novas *et al.*, 2003; Iannone & Cabral, 2006).

Patagonia is a broad region in South America where the climate is generally cold, windy and dry. In the extra-andean

area, precipitation declines from 300 mm in the west to < 150 mm in the east, increasing slightly towards the Atlantic coast (Soriano, 1983).

The incidence, distribution and association of endophytes with native grasses are poorly known (Saikkonen *et al.*, 2000). The presence of many native endophyte-infected grasses broadly distributed in Patagonia and with no recorded toxicity (Somlo *et al.*, 1985; Posse *et al.*, 1996) lead us to hypothesize that the advantages of infection are due to selective pressures other than mammalian herbivory. Therefore, *Bromus setifolius* J. Presl, *Phleum alpinum* L. and *Poa spiciformis* (Steud.) (Hauman & Parodi), perennial and widespread grasses in Patagonia (Gutiérrez & Penseiro, 1998), were selected for this study.

In a previous survey in the Mendoza province, Argentina, endophyte infection levels were estimated in populations of *B. setifolius* at 13 sites in the Andes Mountains (White *et al.*, 2001). A high percentage of infected plants was found in populations located in communities that contained leaf-cutting ants (*Acromyrmex* sp.), supporting the defensive mutualism hypothesis from herbivores.

The objective of the present study was to determine the incidence of the *Neotyphodium* endophyte in *B. setifolius* populations of the Santa Cruz province, and populations of *Ph. alpinum* and *P. spiciformis* of the Tierra del Fuego province, South Patagonia, Argentina, and their association with environmental and soil conditions.

Material and methods

Study areas and plant materials

Patagonia, in the south of South America, is a cool semi-desert (Soriano, 1983). The dryness of the extra-andean Patagonia, characteristic of Santa Cruz, is caused, in part, by precipitation below 300 mm and also by strong winds that cause high evaporation rates (Soriano, 1983). In addition, low mean annual temperature and extreme cold winters create severe restrictions to plant growth (Ravetta & Soriano, 1998). Soils present characteristics mostly related to the arid (Ares *et al.*, 1990). The climate in the Tierra del Fuego province is semiarid to subhumid, with oceanic characteristics (Korembli & Forte Lay, 1991). The precipitation decreases from the southwest to the northeast, away from the Andes range. In the northern extreme it rains 300 mm, while in the south it rains more than 450 mm, per year (Collantes *et al.*, 1989).

Bromus setifolius [nomenclature follows Cámara Hernández (1978) and Matthei (1986)] is a perennial grass native to Patagonia (Zuloaga *et al.*, 1994; Gutiérrez & Penseiro, 1998). *Phleum alpinum* is a perennial grass commonly found in wet soils, throughout the Andean mountains, from Mendoza province to Tierra del Fuego and it presents a wide distribution among the principal ecological areas of this region. The material of *Poa* studied in the present work was previously considered as *P. rigidifolia* Steud. in Giussani & Collantes (1997) and is now considered as *P. spiciformis* in Soreng *et al.*

(2003). This plant is also a perennial grass, found in Tierra del Fuego, the Strait of Magellan, the Falkland Islands and areas adjacent to Chile (Nicora, 1978). In Tierra del Fuego, the latter is restricted to the communities of the Fuegian steppe and is one of the scarce grasses that grow in the *Empetrum rubrum* heathland (murtillares), considered the poorest lands of this region. *Poa* is one of the principal taxa consumed by sheep throughout the year (Posse *et al.*, 1996). There are no records of toxicity to livestock for any of these grasses.

Sampling

The survey was performed in the summers of 1998, 1999 and 2001 for the three host plants.

Bromus setifolius was sampled in 36 sites along a north-west–southeast transect, c. 800 km long, including the main ecological areas of the south of the Santa Cruz province. These areas were established by Oliva *et al.* (2001) after an exhaustive compilation of the existent information, and could be considered a particular combination of soil, climate and vegetation (Table 1). *Phleum alpinum* populations were sampled at the principal vegetation areas of the Tierra del Fuego province: Fuegian steppe, Fuegian ecotone and Fuegian-andean complex. Within the Fuegian ecotone and the Fuegian-andean complex, two plant communities were sampled; deciduous forest (*Nothofagus antarctica*) and evergreen forest (*N. pumilio*) (Moore, 1983). Within the Fuegian steppe we sampled three different plant communities: grasslands, *Chiliodactylon* dense scrub and 'vegas' (drainage network of creeks and valleys covered with hygrophilous vegetation). The populations were distributed along a southwest–northeast transect. Sampling of *P. spiciformis* populations was limited to the Fuegian steppe and to the Fuegian ecotone. Three plant communities, associated to a nutritional gradient, were chosen along the main floristic

Table 1. Characterization of the ecological areas surveyed at south Santa Cruz province (following Oliva *et al.*, 2001)

Ecological area	Extension (Mha)	Altitude (m.a.s.l.)	Annual average temperature (°C)	Average rainfall (mm)	Main plant community where <i>B. setifolius</i> plants were collected	Endophyte incidence ($\bar{x} \pm SD$)
Andean complex	1.76	> 500	5.5–8	> 500	<i>Nothofagus antarctica</i> and <i>N. pumilio</i> forest interspersed by patches of grasses	87.4 ± 15.1 a
Dry Magellanic steppe	1.17	< 300	6–7	170–300	'Coironal fueguino' of <i>Festuca gracillima</i>	1 ± 3.16 c
Humid Magellanic steppe	0.39	0–300	5.5–6.5	200–400	Same as above community with sparse shrubs of <i>Chiliodactylon diffusum</i> and with a high cover of 'murtillares' (<i>Empetrum rubrum</i>)	75 ± 27.8 ab
<i>Junellia</i> scrub	2.83	0–900	6.5–8.5	150–200	Dominated by <i>Junellia tridens</i> (cover 60–70%) and with abundant bare soil	2.2 ± 6.6 c
Subandine grassland	2.18	300–500	7–8	300–500	Graminean steppe dominated by <i>Festuca pallescens</i>	17.6 ± 19.7 bc

Incidence of *Bromus setifolius* populations is based on the presence/absence of endophyte hyphae of 20 plants per population. Means in a column not sharing a common letter are significantly different ($P < 0.05$) according to Dunn (1964).

Table 2. Characterization of the three plant communities chosen along the main floristic gradient of the Fuegian steppe and the Fuegian ecotone in the Tierra del Fuego province (Collantes *et al.*, 1999)

Ecological area	Plant community	Soil nutritional characteristics
A	This is a tussock grassland rich in forbs and graminoids. Diversity and richness are high.	Soils are the most eutrophic of all communities, high in organic matter with a low C/N ratio and rich in Ca ⁺⁺ content.
B	It can be divided into two categories: <i>Chiliotrichum</i> scrub with <i>Empetrum</i> (a 60-cm high dense scrub among which, <i>Empetrum</i> and other grassland components appear), or <i>Festuca-Empetrum</i> grassland (a tussock layer of <i>Festuca</i> and a lower layer of dwarf shrubs -mainly <i>Empetrum</i> -, graminoids and typical acidophilous forbs).	Soil parameters indicate intermediate fertility.
C	A vegetation type of prostrate and cushion shrubs, dominated by <i>Empetrum</i> , floristically very poor.	Soils are very infertile, with high organic material content poorly decomposed (high C/N), low base saturation, low pH and low Ca ⁺⁺ content.

gradient (Collantes *et al.*, 1999): A, *Festuca-Poa* grasslands (without *Empetrum*, neutrophylous); B, *Festuca-Empetrum* communities (with intermediate *Empetrum* cover, slightly acidic and with *Chiliotrichum* and/or *F. gracillima*) and C, communities with high *Empetrum* cover (highly acidic) (Table 2).

Twenty specimens of each host plant were randomly collected from each of the natural populations. Aerial tissues of plants were collected and stored in plastic bags for further laboratory analysis.

Site characterization

At each site, the geographical coordinates and the altitude above sea level (m) were registered using a GPS (Global Positioning system).

Considering the wide distribution of the *B. setifolius* in Santa Cruz and *Ph. alpinum* in Tierra del Fuego, the association between endophyte incidence and environmental characteristics was studied. Five climatic variables were considered in order to analyse the environmental variability within the studied area. These variables were recorded from De Fina (1992), which consists of a 10–30 year compilation of climatic data obtained from the meteorological stations nearest the sampled localities. The variables were (1) warmest month average temperature (°C), (2) coldest month average temperature (°C), (3) annual average rainfall (mm), (4) warmest quarter average rainfall (mm), and (5) coldest quarter average rainfall (mm).

In *B. setifolius* populations, soil samples of the upper horizon (5–15 cm) were taken in 10 sites (1, 4, 5, 6, 7, 8, 18, 22, 23 and 24) that we considered representative from all ecological areas. Samples were subjected to the following analyses [according to Jackson (1981)]: pH in water 1:25; E.C; total C (Walkley–Black); total N (Kjeldahl); P; C.E.C. (ammonium acetate 1 N, pH 7), Ca⁺⁺, Mg⁺⁺, Na⁺ and K+

by the Laboratory of Geological and Edaphological Chemistry, CONICET, Argentina.

Vegetation characterization and presence of ant colonies

At each site where *B. setifolius* was collected, an area of c. 100 m² was sampled to determine the vascular species present and to estimate a value of cover-abundance by means of the Braun-Blanquet scale modified by Westhoff & van der Maarel (1978).

The presence of colonies of leaf-cutting ants in the immediate vicinity was identified to determine whether trends observed in Mendoza province occurred elsewhere in South Patagonia.

Endophyte infection

At least two culms of each plant were assessed for the presence of endophytic mycelia by examination of culm tissue using a light microscope. Endophytic mycelia were visualized by staining tissue scraped from within culms with aniline blue (0.1% aqueous) (Clark *et al.*, 1983). Culms were identified as endophyte-infected if typical nonbranching intercellular mycelia were evident among plant parenchymal tissues.

Because the sample size was limited (20 per population) and therefore may not include infected plants in populations where infection frequencies are low, and also because culm staining may miss hyphae in infected plants, our estimates of endophyte frequency should be considered conservative.

Endophyte isolation

Endophytes were isolated from fresh culm material and seeds. Culms, cut in c. 1-cm-long pieces, and seeds, when available, from endophytic-infected populations were

surface-disinfected. The sterilization was achieved by immersion in 70% ethanol for 1 min, 50% commercial bleach (3% Na-hypochlorite) for 3–5 min and 50% ethanol for 1 min. The pieces and/or seeds were placed on plates with potato dextrose agar (PDA). Plates were incubated at 23 °C. The typical white cottony colonies of *Neotyphodium* emerged after about 4 weeks. Selected strains were transferred to slants, incubated at 23 °C until sufficient growth had occurred and then stored at 4 °C until identification.

Cultures with typical slow growth were examined and observed to confirm whether they fit the *Neotyphodium* type morphology (Morgan-Jones & Gams, 1982) and to observe strain variability. Observations and measurements were taken from fresh material mounted in distilled water, 5% KOH and phloxine for optical microscopy. The isolates were characterized by measuring the lengths and widths of 10 conidia per isolate.

Statistical analysis

Principal component analysis (PCA) was used to characterize sample sites according to climatic variables, latitude, longitude and altitude in *B. setifolius* and *Ph. alpinum*. Data were standardized following Matteucci & Colma (1982) and Crisci & Lopez Armengol (1983). To study the association between the environmental data and the endophyte incidence, an external validation was made by means of the Pearson correlation coefficient to correlate the site scores of the first axis with the scores of incidence. Statistical analyses were performed with the statistical package PC-Ord (McCune, 1991).

Populations of each host plant were grouped into the ecological areas and the differences in the endophyte incidence were tested using the Kruskal–Wallis test. Comparison of means was performed with Dunn (1964).

In *B. setifolius*, the Pearson correlation coefficient was used to estimate the correlation between endophyte incidence and soil nutrients. For vegetation analysis, samples and species were simultaneously classified with the TWINSPAN divisive method (Hill, 1979).

A one-way ANOVA was used to determine differences in the incidence of endophyte populations between plant communities of *P. spiciformis*.

Results

Endophyte infection

In plants of *B. setifolius*, intercellular endophytic mycelia were observed in 15 out of the 36 populations (39%). The endophyte incidence of the populations varied from 0% to 100% (Table 1). The location and the endophyte incidence of each population are shown in Fig. 1. In *Ph. alpinum*, infection was observed in 28 out of the 31 populations

(90%) and the endophyte incidence of the populations varied from 0% to 100%. All the populations of *P. spiciformis* were infected by endophytes and the incidence exhibited a range of 40% to 100%. The location of *Ph. alpinum* and *P. spiciformis* populations are shown in Fig. 2. The sexual *Epichloë* stages were not found in any population sampled.

Detection, isolation and identification of *Neotyphodium* fungi

In *B. setifolius*, 16 isolations were obtained from eight populations. Five of the isolates obtained in the present work were previously studied in a phylogenetic analysis of sequences from variable portions of genes for β -tubulin and translation elongation factor 1- α (tef1) (Gentile *et al.*, 2005). That study suggested that three out of the five isolates were apparently nonhybrid endophytes. In an attempt to find some relation between the hybrid status and the geographic distribution, we observed that the nonhybrid isolates were obtained from different populations located many kilometres away from each other. On the other hand, both a nonhybrid isolate and a hybrid isolate that shared plant community and environmental conditions were obtained from places quite near each other.

In *Ph. alpinum* seven isolates were obtained from three populations while in *P. spiciformis* six isolates were obtained from four populations. All isolates were deposited in the Culture Collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (BAFCcult).

Populations and abiotic factors: correlation with endophyte incidence

Bromus setifolius

As a result of the PCA based on climatic variables, latitude, longitude and altitude, the first three axes accounted for the 92.61% of total variability. Axis I was positively associated with annual and coldest quarter average rainfall. Axis II was positively associated with altitude and longitude and it was negatively associated with latitude (Fig. 3).

The Pearson coefficient ($r = 0.57$; $P = 0.001$) between the scores of axis I and incidence percentage indicates a positive and significant association between the precipitation gradient and endophyte incidence. The amount of variance in endophyte incidence explained by the climate data was $r^2 = 0.325$, or 32.5% of the variation. This percentage is important considering the length of the transect studied. The variables most correlated with endophyte incidence were annual average rainfall, warmest quarter average rainfall and coldest quarter average. This result agrees with the map where populations with high endophyte colonization percentage are located near the Andean Complex or in Cabo Virgenes, where the maritime influence is evident. The only

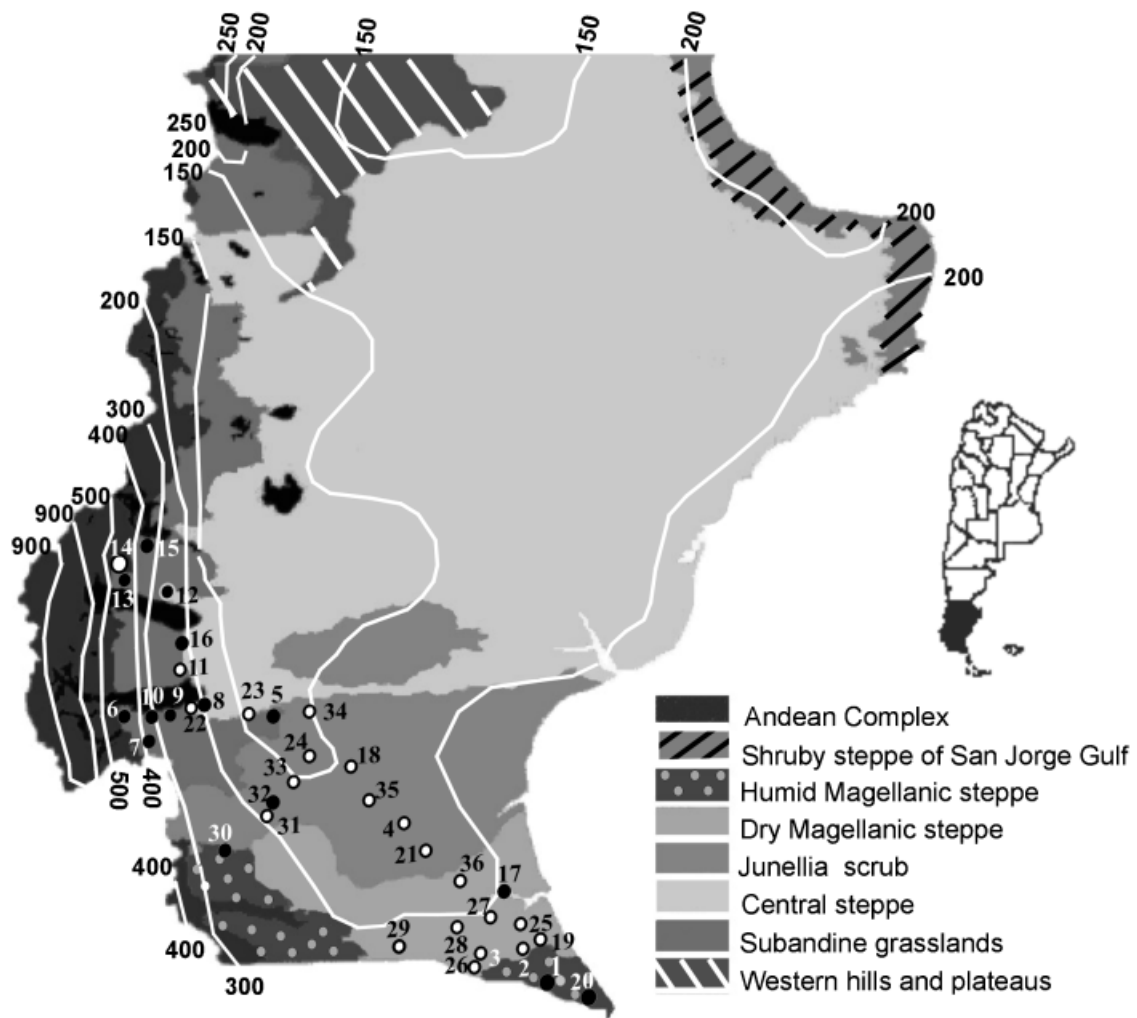


Fig. 1. Geographic location of 36 native populations of *Bromus setifolius*, in Santa Cruz province, Argentina, showing *Neotyphodium* endophytes incidence in relation to ecological areas and isohyets. Black circles are *B. setifolius* endophyte-infected populations, white circles are *B. setifolius* populations free of endophytes. Modified from Oliva et al. (2001).

exception was population 14, associated with a *Nothofagus* forest, which presented 0% endophyte incidence and a high precipitation rate. Endophyte incidence was not significantly correlated with any of the soil parameters analysed.

Phleum alpinum

The statistical analysis recognized the site number 8 as an outlier. This site presents large differences in the values of some of the variables used in this study, such as altitude, rainfall and temperatures. For this reason we excluded this site from the statistical analysis. Endophyte incidence in analysed populations showed an association with environmental variables. Figure 4 shows the display of the populations on the two first ordination axes. The first three axes absorbed 93.3% of the total variance. Axis I was negatively associated with the warmest quarter average rainfall and

latitude. Axis II was highly and negatively associated with altitude and it was positively associated with temperature. Populations from *N. pumilio* forest sites formed a separate group, showed the lowest endophyte incidence and were located at the most humid area. Therefore, the distribution of the populations, as seen in Fig. 4, coincides with a decrease in the precipitation rate together with an increase in the endophyte incidence. The Pearson coefficient ($r=0.476$; $P=0.01$) indicated a positive and significant association between climate and endophyte incidence. The amount of variance in endophyte incidence explained by the climate data was $r^2=0.24$.

Although there is a difference in endophyte incidence between the *N. pumilio* forest and the rest of the plant communities (Fig. 5), differences in endophyte incidence between plant communities were not significant ($H=6.3$; $P=0.177$).

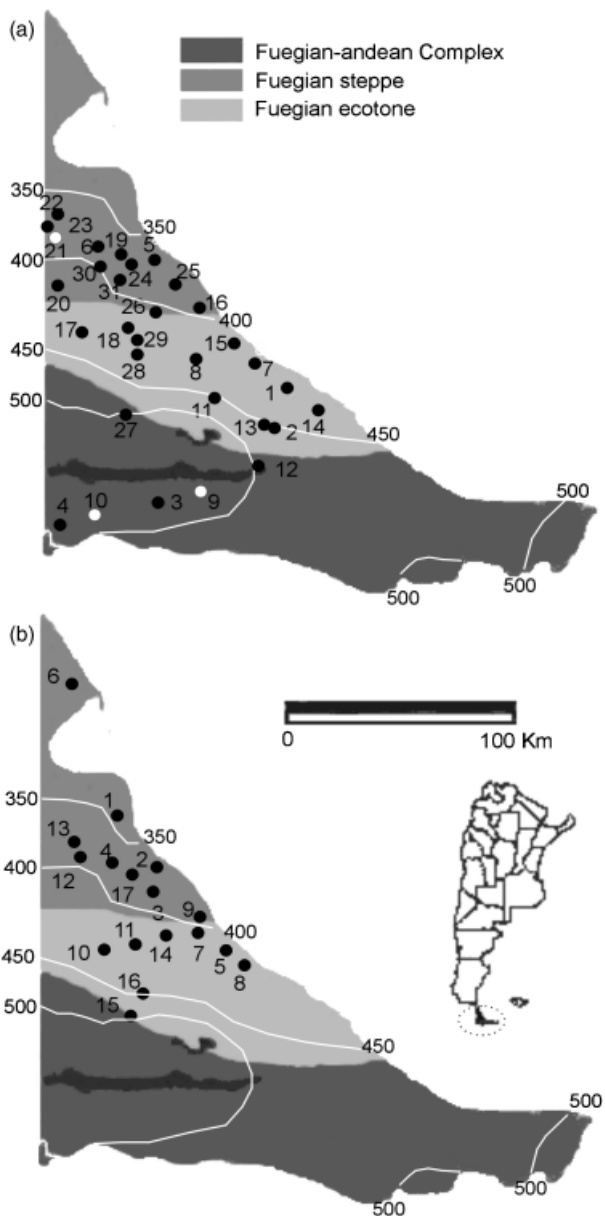


Fig. 2. Geographic location of native populations of *Phleum alpinum* and *Poa spiciformis*, showing the ecological areas and isohyets in Tierra del Fuego province, Argentina. (a) *Phleum alpinum* populations. (b) *Poa spiciformis* populations. Black circles are endophyte-infected populations, white circles are populations free of endophytes. Modified from Oliva *et al.* (2001).

Poa spiciformis

Unlike that observed in *Ph. alpinum*, differences in endophyte incidence between plant communities in *P. spiciformis* were significant ($F=7.798$; $P=0.02$).

Comparisons of means showed the presence of two groups. Endophyte incidence was significantly different between grasslands and ‘extreme murtillares’; however, incidence values observed in *Festuca-Empetrum* murtillares

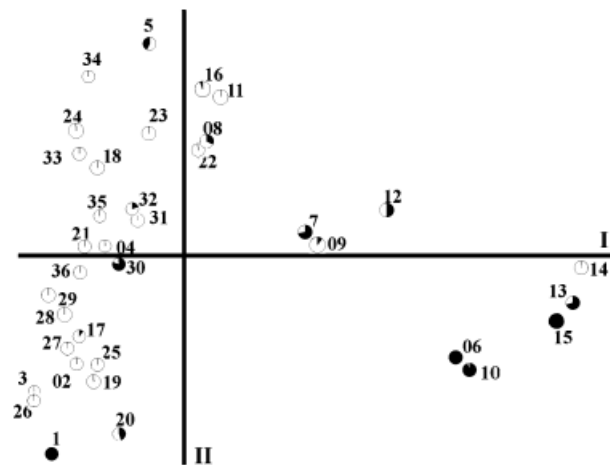


Fig. 3. Principal component analysis (PCA) ordination diagram of 36 populations of *Bromus setifolius* and environmental data. The proportion of each circle shaded in black indicates the endophyte incidence.

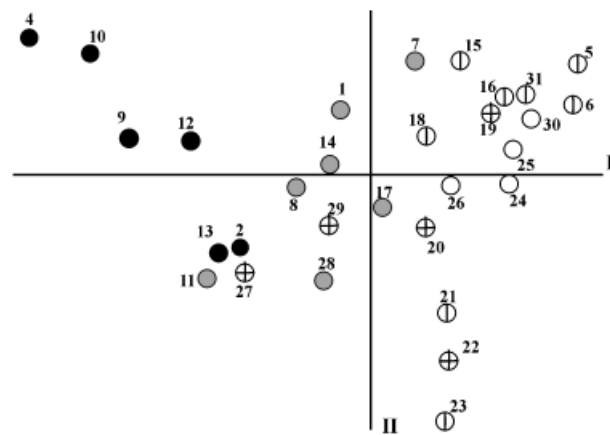


Fig. 4. Principal component analysis (PCA) ordination diagram of 30 populations of *Phleum alpinum* and environmental data. Full black circles represent *Nothofagus pumilio* forest, full grey circles *N. antarctica* forest, open circles *Chiliodrion* scrub, crossed circles ‘vegas’, vertically intersected circles grassland.

did not differ significantly from the other two plant communities (Fig. 6).

Association of endophyte incidence with vegetation and ant colonies

Five types of communities were obtained from the TWINSPAN analysis in *B. setifolius*. These communities agree with dominant communities of the ecological areas recognized by Oliva *et al.* (2001). Table 1 shows the samples (populations), followed by the endophyte colonization percentage, grouped into the ecological areas.

Populations grouped into the ‘Mata negra’ scrub (*Junellia tridens*) and the Dry Magellanic steppe are almost free of endophyte. Subandine grassland populations presented

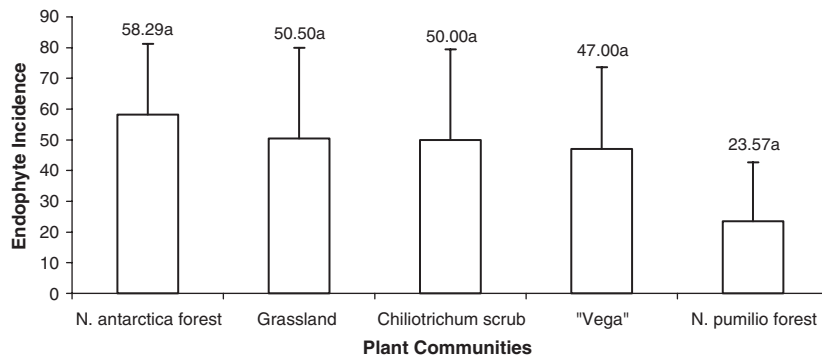


Fig. 5. Mean (SE) endophyte incidence of *Phleum alpinum* populations in five different plant communities sampled in Tierra del Fuego. Different letters above bars indicate significant differences in endophyte incidence between plant communities (Kruskal–Wallis test, $P=0.05$).

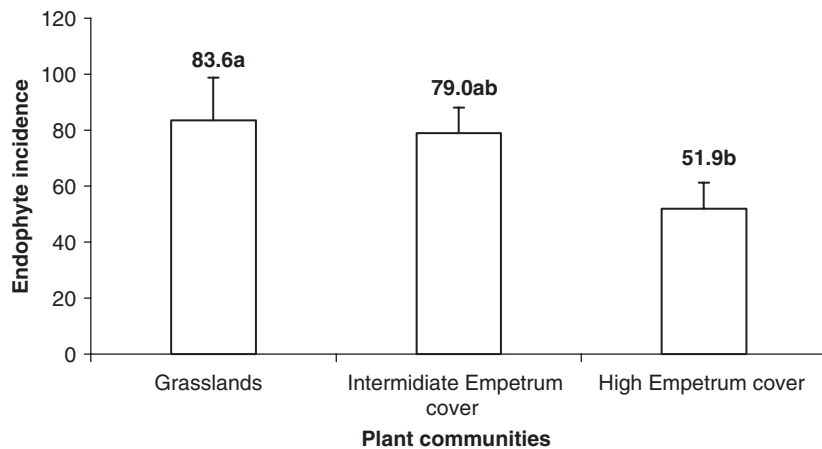


Fig. 6. Mean (SE) endophyte incidence of *Poa spiciformis* populations in three different plant communities: grasslands, *Festuca-Empetrum* murtillares and 'extreme murtillares'. Different letters above bars indicate significant differences in endophyte incidence between plant communities (Kruskal–Wallis test, $P=0.05$).

intermediate endophyte incidence values. Populations belonging to the Humid Magellanic Steppe and the Andean Complex showed incidence percentages near 100. These differences in endophyte incidence were significant ($H=25.4$; $P < 0.0001$), and comparisons of means showed three groups (Table 1). Plant communities were associated with precipitation and communities with a high rainfall rate presented endophyte-infected populations.

We did not register the presence of leaf-cutting ants or any other insect in high proportions to consider their association to the endophyte incidence.

Discussion

The present work makes an important contribution to our understanding of the variation in incidence and distribution of *Neotyphodium* endophytes in populations of wild grasses. There are a few studies in the subject. In two of them the incidence was obtained from seeds, instead of plants, and may not be representative of the real incidence in the natural populations (Lewis *et al.*, 1997; Clement *et al.*, 2001). In addition, in one of the studies (Clement *et al.*, 2001) the plant host was *F. arundinacea*, an agronomic grass, and in the other (Lewis *et al.*, 1997) the plant material was *Lolium* spp., also agronomically important. Zabalgoeazcoa *et al.* (1999) carried out a survey in 27 populations of *F. rubra* but

the incidence was determined only in six selected populations. To our knowledge, the present work is the most extensive yet published for the incidence and distribution of *Neotyphodium* endophytes in populations of wild grasses and its association with environmental parameters.

More than half of the *B. setifolius* populations studied were endophyte-free. However, in *Ph. alpinum* and *P. spiciformis*, all populations sampled were endophyte-infected with only three exceptions. The frequency of endophyte infection could be highly variable between populations from different ecological areas due to host genotypes and the heterogeneity in selective forces expected among habitats (Saikkonen *et al.*, 2002).

Endophyte-colonized grasses express a range of adaptations to biotic (Latch, 1993) and abiotic stresses (West, 1994). Increased drought resistance has been proposed in some endophyte-infected grasses as one of the benefits that the host could obtain from the association (Malinowski & Belesky, 2000). In contrast to these results, other authors have obtained a large variability in host responses to endophyte infection (Morse *et al.*, 2002; Faeth & Sullivan, 2003; Cheplick, 2004).

White *et al.* (2001) studied *B. setifolius* populations, in the Mendoza province, Argentina, and found that the association between host and endophyte is positively correlated with ants. In the present study we found that the association

seems to be related to drought. Populations located at the driest regions (Dry Magellanic steppe and *Junellia* scrub) were almost free of endophytes. The populations located at the more humid regions, such as the *Nothofagus* forest border or in Cabo Virgenes, in the Atlantic Ocean, showed the highest endophyte colonization rate. Moreover, populations found in areas with an intermediate precipitation rate (Subandine grassland) showed an intermediate endophyte colonization level. Site 14, the only population found in the forest presenting a high precipitation rate (784 mm), was the exception since it showed 0% of endophyte incidence. Similar results were found in *Ph. alpinum* populations associated to *Nothofagus* forest in Tierra del Fuego (see below). The different responses obtained in *B. setifolius* from the assays previously performed under controlled environmental conditions (Novas *et al.*, 2003) and those from the field observations suggest that in low growth conditions, such as those of the extra-andean Patagonia, the association is not favoured, leading to a decrease in the endophyte frequency of infection or even to the complete loss of the association.

In *P. spiciformis*, the association between endophyte incidence and plant communities was analysed considering the *Empetrum* cover as a stressful factor in soil. Communities with a high dominance of *E. rubrum* are related to very infertile soils (Collantes *et al.*, 1999).

Endophyte incidence was highest in the grasslands, lowest in the communities with high *Empetrum* cover, and intermediate in the communities with intermediate *Empetrum* cover. Soil productivity might modify the outcome of the grass-endophyte interaction if nutrients are limited (Cheplick *et al.*, 1989; Faeth *et al.*, 2004), and as suggested by Ahlholm *et al.* (2002) the cost of endophytes appears to outweigh their benefits under conditions of limited resources. A similar effect was observed in populations of *P. spiciformis* located in plant communities with a high cover of *E. rubrum*. However, even under the most stressful conditions, the association between endophytes and *P. spiciformis* was present. This host is one of the scarce grasses that grows in the *E. rubrum* heathland, so its capacity to recover eroded soils should be studied.

In *Ph. alpinum* endophyte incidence was negatively correlated with the precipitation rate. Populations associated with *N. pumilio* forests located at the most humid areas showed the lowest incidence values, while those located at the most xeric areas presented the highest endophyte infection. The precipitation rate is higher in Tierra del Fuego than in Santa Cruz (Oliva *et al.*, 2001), and probably this factor is not as limiting for plant productivity in Tierra del Fuego as it is in Santa Cruz. Therefore, endophytes might enhance drought tolerance in *Ph. alpinum* populations of Tierra del Fuego.

The *N. pumilio* forest is a community characterized by poor plant diversity and a low rate of growth. There are

other environmental variables associated with this community, such as light and soil acidification, that we did not study that might be affecting the endophyte incidence. On one hand, the amount of light that reaches the soil is scarce due to the height and density of the trees. And on the other hand, soil acidification increases towards the south, where the evergreen forest is located, probably as a result of high drainage and slower organic matter decomposition (Godeas *et al.*, 1993). As mentioned earlier, the *B. setifolius* population sampled in the forest showed 0% of endophyte incidence. In plants growing under these stressful conditions, the association may not be favoured, but more forest communities should be sampled to corroborate this hypothesis.

In a prior study which investigated the phylogeny of *Neotyphodium* spp. from native grass species throughout Argentina, five of the isolates of *B. setifolius* obtained in the present work were used (Gentile *et al.*, 2005). That study suggested that three out of the five isolates were apparently nonhybrid endophytes (Gentile *et al.*, 2005). We did not find any correlation between the hybrid and nonhybrid isolates and their geographical origin or the environmental characteristics analysed. To corroborate these results it would be necessary to perform a more intensive study.

The present results suggest that the distribution of native grass populations infected with *Neotyphodium* endophytes depend, in many cases, on the environmental conditions, in particular of the type and intensity of stress. In a biotic and abiotic heterogeneous area, the results of the survey of a host species in relation with the endophyte incidence could allow us to determine whether the symbiosis is beneficial or detrimental. The *Neotyphodium*-grass association may be affected by the plant genotype and the fungal strain as well as by both geographic and temporary environmental and biotic factors. In the present study the populations were sampled more than once during different years and we did not observe a significant change in the endophyte infection frequency. As an example, to maintain the beneficial characteristics of the association in *B. setifolius*, considered a good forage grass and not toxic to livestock, it should be managed only in regions where the annual rainfall rate exceeds 290 mm, where we observed that the association is sustained in field conditions. This direct observation of the symbiosis in the field gives us more reliable data than those obtained in experiments performed under controlled environmental conditions. However, these types of experiments are important to corroborate field surveys.

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