The distribution and incidence of *Neotyphodium* endophytes in native grasses from Argentina and its association with environmental factors

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Abstract

We examined the occurrence of *Neotyphodium* endophytes in native grasses from Argentina using both herbarium material and plants collected from the field. *Neotyphodium* endophytes were detected in 34 out of more than 150 host species examined throughout the country. The incidence of infection was highly variable within many of the species. We examined the relationship between incidence of endophyte and environmental conditions in four host species, *Bromus setifolius* from Santa Cruz, *Phleum alpinum* and *Poa rigidifolia* from Tierra del Fuego and *Poa bonariensis* from the Pampean region. The results suggest that each endophyte-host association seems to be affected in different ways by environmental factors. *B. setifolius* only contained endophyte in moist rather than dry regions, while in contrast, *Ph. alpinum* showed the highest endophyte incidence in the driest sites. Endophyte incidence in *P. rigidifolia* was highest where soil fertility was high, and *P. bonariensis* contained the most endophyte in high pH rather than low pH soils. Field observations suggest that in poor growing conditions, such as those of the extra-Andean Patagonia, or highly acidic soils, the association is not favoured leading to a decrease in the endophyte frequency of infection or even to the complete loss of the association.

Keywords: *Bromus setifolius*, native grasses, *Phleum alpinum*, *Poa bonariensis*, *Poa rigidifolia*, South America.

Introduction

Both native and naturalised grasses are widespread in Argentina and are an important source of grazing for cattle, as well as providing a genetic resource for further forage breeding. The role of an endophytic fungus in *Festuca hieronymii* as the cause of mammalian toxicosis has been known in Argentina since the beginning of last century (Rivas & Zanolli 1909).

In the last 20 years many cool-season grasses have been found to be colonised by endophytes in Argentina. Endophyte strains have been isolated from three toxic grass species and 19 from species with no known toxicity problems (Bertoni *et al.* 1993; White *et al.* 1996). Cabral *et al.* (1999) analysed isozymes and DNA sequences in endophyte isolates from the three toxic grasses and as a result a new species was named *Neotyphodium tembladerae*. Relationships among *Neotyphodium* isolates from 10 native grasses were studied by phylogenetic analysis. Most of the Argentine endophytes seem to be interespecific hybrids of *Epichloë festucae* and *E. typhina* (Gentile *et al.* 2005). Until now, the sexual stage, *Epichloë* sp., has not been found in Argentina or elsewhere in South America (Gentile *et al.* 2005).

Here we review the research performed on *Neotyphodium* endophyte distribution and incidence in native grasses from Argentina. We also report on the association between *Neotyphodium* incidence and biotic and abiotic parameters in populations of several native hosts located in different ecological regions of Argentina.

Methods

Endophyte survey and isolation

A survey of the endophytes of native grasses was carried out on herbarium plant material and by analysing plants in natural populations throughout the country, including the main ecological regions. Endophytes were isolated on PDA plates from surface sterilised fresh leaf sheaths, culms or seeds. Cultures with typical slow growth were examined and macro- and micro-morphological features were observed to confirm whether they fitted in *Neotyphodium* typing.

Association with environmental parameters

For this study we have analysed four grass species found in environments that differed in ecological characteristics (*Bromus setifolius*, *Phleum alpinum*, *Poa rigidifolia* and *Poa bonariensis*). The first three species are distributed in Patagonia, a broad ecological region in South America where the climate is generally cold, windy and dry. *B. setifolius* grows in Santa Cruz province while *Ph. alpinum* and *P. rigidifolia* in Tierra del Fuego. *P. bonariensis* occurs in temperate and humid grasslands in the Pampean region.

The sampling criteria were to collect from different populations of each host species in order to examine all the environments inhabited by each one. Twenty specimens of each host plant were randomly collected from each population. At least two culms or tillers of each plant were microscopically examined to detect the presence of endophytic mycelia.

B. setifolius was sampled in 36 sites along an 800 km SE-NW transect, covering the main ecological areas of the south Santa Cruz province.

Thirty one populations of *Ph. alpinum* were sampled, along a S-N transect, in the principal vegetation areas of Tierra del Fuego province.

Sampling of *P. rigidifolia* (17 populations) was restricted to the Fuegian steppe in Tierra del Fuego, where three types of plant communities were chosen along the main floristic gradient and associated with a nutritional gradient (Collantes *et al.* 1999):

- A- *Festuca-Poa* grasslands (neutrophylous soils);
- B- *Festuca-Empetrum* communities (slightly acidic soils) and
- C- Communities with high *Empetrum* cover (highly acidic soils).

In *P. bonariensis* the endophyte incidence was studied in 20 populations throughout all of its distributed range in Argentina.

For *B. setifolius* and *Ph. alpinum*, the variables analysed were: average temperature of the warmest month (°C), average temperature of the coldest month (°C), annual average rainfall (mm), average rainfall (mm) in the warmest quarter, and average rainfall (mm) in the coldest quarter.

Statistical analysis

Principal component analysis (PCA) was used to characterise sample site data of *B. setifolius* and *Ph. alpinum*. Association between environmental data and endophyte incidence was made by means of the Pearson correlation coefficient (PC).

Results and Discussion

Fungal and host diversity

More than 150 host species were examined for endophyte presence. Thirty four host species, mostly in the genera *Poa* and *Festuca*, were found to be infected with *Neotyphodium* endophytes. Only three of the grasses are known to be toxic to cattle and sexual forms in the genus *Epichle* were not found in this survey. Host species are mostly distributed along cordillera

de Los Andes although many others are found in rain forest zones and grasslands in the Pampas and coastal regions. Endophytes were isolated from 19 host species (Table 1). Most of the endophytes isolated fitted the characteristics of *Neotyphodium*. Considerable morphological variability was observed between endophytes isolated from different host species or from different populations of some species. Although *Acremonium* species were isolated too, they were not considered in the analysis.

Table 1 Native grasses species from Argentina analysed for endophyte infection. Endophyte identity was established by means of morphological and molecular techniques (phylogenetic analysis of sequences from variable portions of genes for β -tubulin (*tub2*) and translation elongation factor 1- α (*tef1*)).

Host	Toxicity	Endophyte
<i>Briza subaristata</i> - like		<i>Neotyphodium</i> sp. ^c
<i>Bromus auleticus</i>		<i>Neotyphodium</i> sp. ^b
<i>Bromus brachyanthera</i>		<i>Neotyphodium</i> sp. ^c
<i>Bromus setifolius</i>		<i>Neotyphodium</i> sp. ^b
<i>Festuca argentina</i>	+	<i>Neotyphodium tembladerae</i> ^{ab}
<i>Festuca dissitiflora</i>	+	not isolated
<i>Festuca erecta</i>		not isolated
<i>Festuca fiebrigii</i>		not isolated
<i>Festuca fimbriata</i>		<i>Neotyphodium</i> sp. ^c
<i>Festuca hieronymi</i>	+	<i>Neotyphodium tembladerae</i> ^{ab}
<i>Festuca magellanica</i>		<i>Neotyphodium</i> sp. ^{bd}
<i>Festuca parodi</i>		not isolated
<i>Festuca simpliciuscula</i>		not isolated
<i>Festuca superba</i>		<i>Neotyphodium</i> sp. ^b
<i>Festuca tucumanica</i>		not isolated
<i>Festuca ulochaeta</i>		not isolated
<i>Melica macra</i>		not isolated
<i>Melica stuckertii</i>		<i>Neotyphodium</i> sp. ^b
<i>Phleum alpinum</i>		<i>Neotyphodium</i> sp. ^b
<i>Poa alopecurus</i>		not isolated
<i>Poa bergii</i>		<i>Neotyphodium</i> sp. ^c
<i>Poa gymnatha</i>		not isolated
<i>Poa bonariensis</i>		<i>Neotyphodium</i> sp. ^c
<i>Poa hieronymi</i>		not isolated
<i>Poa huecu</i>	+	<i>Neotyphodium tembladerae</i> ^{ab}
<i>Poa lanigera</i>		<i>Neotyphodium</i> sp. ^c
<i>Poa lanuginosa</i>		<i>Neotyphodium</i> sp. ^c
<i>Poa durifolia</i>		<i>Neotyphodium</i> sp. ^c
<i>Poa ligularis</i> - like		<i>Neotyphodium</i> sp. ^c
<i>Poa lilloi</i>		not isolated
<i>Poa plicata</i>		not isolated
<i>Poa rigidifolia</i>		<i>Neotyphodium</i> sp. ^b
<i>Poa stuckertii</i>		not isolated

^a Cabral *et al.* 1999. ^b Gentile *et al.* 2005. ^c Morphological characterisation ^d also recorded near Puerto Natales, Chile, Stewart unpublished

Incidence of *Bromus setifolius* and *Phleum alpinum*

In *B. setifolius* PCA and PC ($r=0.57$; $P=0.001$) (not shown) between the scores of axis I and incidence of endophyte indicates a positive and significant association between the precipitation gradient and endophyte incidence, it is also observed in the

histogram (Fig. 1). The populations from more humid regions showed the highest endophyte infection rate, populations found in areas with an intermediate precipitation rate showed an intermediate incidence, and populations from extremely dry regions were free of endophytes.

Figure 1 Mean endophyte incidence of *Bromus setifolius* populations in five different plant communities: Andean complex, Sub-Andine grasslands, *Junellia* scrub, dry and humid Magellanic steppe. Different letters above bars indicate significant differences in endophyte incidence between plant communities (Kruskal-Wallis test, $P=0.05$).

Ph. alpinum endophyte incidence exhibited a different pattern (PC ($r=0.48$; $P=0.01$)). Populations associated with *Nothofagus pumilio* forests occurring in the most humid areas showed the lowest incidence, while those located at the driest sites exhibited the highest endophyte infection rates (Fig. 2).

Figure 2 Principal Component Analysis (PCA) ordination diagram of endophyte incidence of 30 populations of *Phleum alpinum* and precipitation gradient (Axis I). Full black circles represents *Nothofagus pumilio* forest; full grey circles *N. antarctica* forest; open circles *Chiliotrichum* scrub; crossed circles “vegas” communities (drainage network of creeks and valleys covered with hygrophilous vegetation); vertically intersected circles grassland.

However, it should be noted that the dry sites where *Ph. alpinum* occurs in Tierra del Fuego are not as dry as those extremely dry sites where *B. setifolius* occurs in Santa Cruz (Oliva *et al.* 2001). Therefore, endophytes may enhance moderate drought tolerance in *Ph. alpinum* populations of Tierra del Fuego and provide no advantage to *B. setifolius* in the extremely dry areas of Santa Cruz. However, we cannot discount the possibility that other environmental variables, such as light and soil acidification (Godeas *et al.* 1993) may also play a role in determining the incidence of endophyte in *Ph. alpinum*, as these were not studied.

Incidence of *Poa rigidifolia* and *Poa bonariensis*

In *P. rigidifolia* endophyte incidence was the highest in the grasslands and lowest in the communities with high *Empetrum rubrum* cover (Fig. 3). These communities have infertile soils, with high organic matter content, poorly decomposed, low base

saturation and $\text{pH}<6$ (Collantes *et al.* 1999). Therefore, in *P. rigidifolia* endophyte incidence was associated with higher soil fertility. Soil productivity is known to modify the outcome of the grass-endophyte interaction if nutrients are limiting (Cheplick *et al.* 1989; Ahlholm *et al.* 2002; Faeth *et al.* 2004).

In *P. bonariensis* endophytes were detected in those populations associated with *Celtis tala* forest, characterised for alkaline soils ($\text{pH}\approx 7.5$). Endophyte incidence in these populations ranged from 20 to 100%. Populations with acidic soils $\text{pH}<6$ were endophyte free.

Figure 3 Mean endophyte incidence of *Poa rigidifolia* populations in three different plant communities: the more fertile grasslands, *Festuca-Empetrum* mutillares and the infertile “extreme mutillares”. Different letters above bars indicate significant differences in endophyte incidence between plant communities (Kruskal-Wallis test, $P=0.05$).

Conclusion

To our knowledge this work presents the most extensive list of *Neotyphodium* endophytes in native grasses in South America. Furthermore, it represents an important and thorough study of incidence and distribution.

Our results suggest an important effect of environmental conditions on the incidence of endophytes in host species studied. Every endophyte-host associations seemed to be affected in different ways by environmental factors. Field observations suggest that in poor growing conditions, such as those of the extra-Andean Patagonia, or in highly acidic soils, the association is not favoured leading to a decrease in the endophyte frequency of infection or even to the complete loss of the association, as observed in other grasses (Faeth 2002; Faeth & Bultman 2002).

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