

Effects of the *Neotyphodium* endophyte status on plant performance of *Bromus auleticus*, a wild native grass from South America

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Abstract

In this work, the hypothesis that endophytes increase plant performance of *Bromus auleticus*, a native grass from South America was tested. Seed weight, seedling emergence, development, and plant vegetative propagation were compared in plants from uninfected (E-), partially infected (E+/-) or infected (E+) seed lots. The hypothesis that endophytes confer resistance to defoliation in one-year-old plants was also tested. Seed lots were collected in different grass populations in the Pampean region in Argentina. E+ lots presented both lighter seeds and lighter and shorter ten-day-old seedlings than E+/- and E- lots. E+ and E+/- lots presented higher percentage and rate of emergence, as well as less time of emergence, than E- seed lots. Plants from E+ lots produced more tillers in a year than E+/- and E- plants. Resistance to defoliation was analysed in plants from one E+ and one E- lot. Infected plants were more resistant to defoliation than E- plants. Our results allowed a preliminary characterisation of this endophyte-plant association. Plants from E+ and E+/- lots presented some advantageous characteristics with regards to seed dispersion, vegetative propagation and resistance to defoliation.

Keywords: *Neotyphodium*, *Bromus auleticus*, symbiosis, plant development, regrowth

1. Introduction

The endophytes in the genus *Neotyphodium* Glenn, Bacon & Hanlin are fungal symbionts growing without causing symptoms in aerial structures in quite a number of cool-season grasses. These fungi are closely related to asexual forms of the genus *Epichloë* Tul. (Clavicipitales) (Glenn et al., 1996).

Whereas *Epichloë* endophytes produce sexual stromata with ascospores on host plants preventing inflorescence development (choke disease), *Neotyphodium*, the asexual form of endophytes, are transmitted vertically via seeds without affecting sexual reproduction of their hosts (Schardl, 2001). Therefore, the grass-*Neotyphodium* interactions are considered to be mutualistic or neutral symbioses while *Epichloë*-grass interaction is considered to be parasitic (Schardl et al., 2004). However, in some associations, *Epichloë* can be beneficial for the plants (Pan and Clay, 2002) and *Neotyphodium* detrimental (Faeth et al., 2004).

As the endophyte makes use of sugars and amino acids in the plant apoplast for its growth (Lam et al., 1994; Lindstrom and Belanger, 1994), the maintenance of such non-pathogenic association would require some benefits for the host plant.

Numerous studies have been conducted in order to determine the putative advantages of these interactions. However, most of these studies have been performed on a few genotypes (Kentucky 31 cultivar) of *Festuca arundinacea* Schreb. (Saikkonen, 2000). Resistance to herbivores is mentioned as the main factor for the maintenance of these plant-endophyte interactions (Clay et al., 1993; Clay and Schardl, 2002), since endophytes produce lolines and peramines, principally active against insects, and indole diterpene and ergot alkaloids, mainly active against mammalian or vertebrate herbivores (Schardl and Phillips, 1997). These symbioses have also been shown to enhance growth (Marks et al., 1991; Clay and Holan, 1999), regrowth after defoliation, and drought resistance of the hosts (Clay and Schardl, 2002). However, all of these effects depend on the interactions among fungal and host genotypes, soil nutrient levels and environmental conditions (Hill et al., 1990; Cheplick, 1998; Belesky and

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Malinowski, 2000; Cheplick et al., 2000; Ahlholm et al., 2002). In natural populations of *Festuca arizonica* Vasey, *Neotyphodium* endophytes do not confer resistance to herbivory (Saikkonen et al., 1999), and they are considered to be parasitic (Faeth et al., 2004). However, under particular conditions, endophyte-infected plants produce more biomass than uninfected ones (Morse et al., 2002). Because of the wide range of responses observed in different plant-endophyte systems, these associations are considered to span continuum from mutualism to antagonism (Saikkonen et al., 1998; Schardl et al. 2004).

In Argentina, *Neotyphodium* endophytes have been detected in numerous native grasses (Bertoni et al., 1993; White et al., 1996; Lugo et al., 1998; White et al., 2001; Gentile et al., 2005). Most of these grasses have not been reported as toxic to cattle in spite of being associated with endophytes. However, *Festuca hieronymi* Heckel, *Poa huecu* Parodi and *Festuca argentina* Parodi infected with *N. tembladerae* Cabral & White are recognised as toxic to cattle (Cabral et al., 1999). In *Bromus setifolius* Presl. the endophytes have been shown to protect the plants against leaf-cutting ants (White et al., 2001).

Recently in our laboratory, *Neotyphodium* endophytes have been detected in *Bromus auleticus* Trin., a native perennial grass inhabiting grasslands of Argentina, Uruguay and southern Brazil. Because of its high forage productivity, richness in proteins, persistence in fields and drought resistance (Moraes and Oliveira, 1990; De Battista and Costa, 1998; Millot, 1999), this grass species is considered as excellent forage, and is being genetically improved, and some cultivars are being commercialised as forage in Uruguay (Millot, 2001).

The aim of this work was to characterise the association between the widespread native, non-toxic grass species *B. auleticus* and its *Neotyphodium* endophytes. To achieve this objective, we used plants from lots of seeds differing in endophyte incidence collected in natural populations. Seed weight, seedling emergence and growth (as a measure of their potential dispersion), colonisation and establishment performance were analysed. Tiller number (as a measure of vegetative propagation) and regrowth capacity after defoliation (as a way of simulated herbivory) were measured in one-year-old plants.

2. Materials and Methods

Plant material

Seed lots collected from natural populations in January 2000 were provided by the EEA-INTA-Anguill germplasm bank. Populations were located in a 150-km diameter area in the north-east of the province of La Pampa, in the Pampean region (Argentina).

Seeds from the E- lots tested were obtained from plants belonging to old accessions from wild populations in La

Pampa which had been stored for a long period. Seeds of these old accessions were sowed in 1999 to collect the seeds used in this work in January 2000. Preliminary studies performed in our laboratory have shown that all the wild populations of *B. auleticus* are infected by *Neotyphodium* endophytes (data not shown). Therefore, we hypothesise that these accessions were originally E+, but they lost the endophytes during the storage.

Incidence of endophytes in each lot was established after analysing 20 seeds and 30 seedlings by aniline blue staining and microscopic observation (Clark et al., 1983).

To compare characteristics associated with the incidence of endophytes, ten lots with: 0% of the seeds infected by endophytes (E-), 40–60% of the seeds infected (E+/-), and 80–100% of the seeds infected (E+) were chosen for the different assays.

The INTA-Anguill accession number and endophyte infection level of each lot are presented along the text in figures and tables together with the results of different assays.

Seed weight determination

Weights were obtained from random samples of 100 filled seeds (cariopsis with palea and lemma) per lot from five lots (two E- (1037 and 1043, 0% infected), one E+/- (5211, 60% infected), and two E+ (5198 and 5216, 100% infected)). An electronic balance was used for weight determination. Seed weight was compared by means of a one-way analysis of variance (ANOVA) between the lots and Tukey test was conducted to compare the means.

Seedling emergence tests

Two assays were carried out to compare the emergence of plants from lots differing in endophyte infection level.

In the first assay, seven lots (two E- (1036 and 1043), one E+/- (2034), and four E+ (2001, 5234, 5274 and 5275)) were compared. Thirty seeds of each population were planted in 18 × 25 × 5 cm trays filled with expanded silicon. The trays were placed in an environmental growth chamber with a diurnal light cycle (12h-light/12h-dark/23±1°C) and soil moisture was kept to field capacity by means of daily watering. In the second assay, weighed seeds from the E- 1043, E+/- 5211 and E+ 5198 lots were sowed in 30 × 50 × 7 cm trays. Soil and growth conditions were the same as in the first assay, except that temperature was kept at 29±1°C. In both assays, the number of emerging seedlings was daily recorded until new seedlings ceased to appear.

Percentage of emergence (PE) ($PE = \sum n/N$), mean time of emergence ($T = \sum tn/\sum n$), and emergence rate ($ER = \sum n/\sum tn$) (where t is the number of days since sowing, n the number of seedlings emerging this day and N the number of sowed seeds) were determined as indicated in Peretti (1994) and Bewley and Black (1994). Chi square test and contrasts

(Siegel, 1956) were utilised to compare the PE (Clay, 1987). One-way analysis of variance (ANOVA) and Tukey post-hoc test for unequal N were used to compare mean times of emergence. Data were log transformed to comply with ANOVA assumptions.

Seedling development and vegetative propagation assays

Sixty randomly chosen 10-day-old seedlings, emerged from each of the three lots used in the assay of emergence at 29°C, were carefully removed from the soil, rinsed in tap water to clean the roots, and blot dried. For each plant, fresh weight, total length, root and shoot length, shoot/root length ratio, and number of fully expanded leaves were determined. Data were analysed by means of a one-way (infection level) ANOVA and Tukey contrast.

After being measured, each plant was planted in 7.5l pots filled with a soil mixture (garden soil:vermiculite, 1:1). Pots were randomly arranged in a greenhouse, in order to allow plants to grow for one year.

Vegetative propagation was periodically measured as the number of tillers present in each plant from E+ and E- lots. The number of tillers in plants from the E+/- lot was measured for six months. Each pot was watered to field capacity three times a week. Commercial fertiliser (500 cm³ of 15:15:15 N P K) was monthly applied as a 2.5-g/l suspension.

Data were subjected to a repeated-measures analysis of variance (ANOVA) performed on the E+ and E- lots, with lot (endophyte level, E) and plant age (A) as main factors.

Regrowth capacity

When the plants used in the development assay were one year old, plant regrowth capacity and mortality were evaluated. Twenty plants from the E+ (5198) lot and other 20 from the E- (1043) lot were randomly chosen. Plants were clipped 5 cm above the substrate level (this harvested biomass was not considered in the assay). From this moment, plants were clipped four times (named C1, C2, C3, C4) allowing a regrowth period of two weeks between clippings.

Leaf biomass collected after each regrowth period was oven dried for 48 hours at 80°C and the harvested leaf dry matter (DM) of each plant was determined. Relative growth rate (RGR) for each regrowth period was calculated as a logarithmic function of the ratio of DM produced in two consecutive regrowth periods divided by the number of days in the growth interval: $RGR = \ln(DM_n/DM_{n-1})/14$. Length of longest leaf, number of living tillers, and plant mortality were determined before each clipping.

Repeated-measures analysis of variance (ANOVA) was used to examine the effects of main factors (endophyte level in the population (E), and clipping (C)) and their interactions on measured variables. Tests comparing means between lots in each clipping or regrowth period were

performed according to the Newman-Keuls procedure (Winer, 1971). Because several E- plants died during the assay, statistical analysis was performed over sixteen plants of each population. Fisher's exact test was performed to analyse plant mortality. All data sets satisfied the assumptions of the repeated-measures ANOVA.

The minimum and maximum greenhouse temperature (daily recorded along the year the research was carried out) were 5°C and 23°C in winter and 23°C and 39°C in summer, respectively.

The incidence of endophytes in the lots studied in the development and regrowth assays was confirmed (when plants were six-month-old) by analysing the presence of endophytes in sheaths of one tiller in every plants of each lot.

3. Results

Seed weight

Seeds from the E+ lots were 14% and 8% lighter than those from the E- and E+/- lots, respectively ($P < 0.001$) (Fig. 1). Tukey comparison showed three significantly different groups, one including both E+ lots, one including the E+/- lot and one of the E- lots, and another one including both E- lots.

Seedling emergence

In the assay performed at 23°C, seedling emergence started between the sixth and the seventh day after sowing in the E+ and E+/- lots, and between the eighth and ninth day in the E- lots. Percentage of emergence (PE) ranged

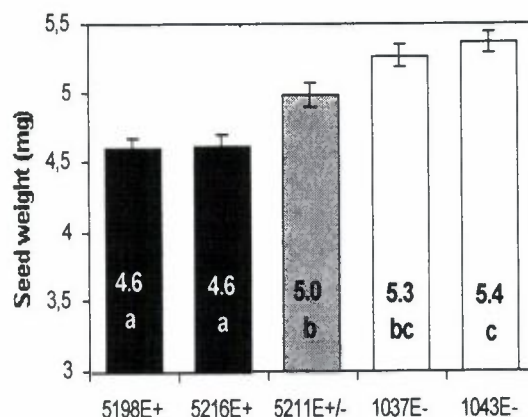


Figure 1. Mean (\pm S.E.) seed weight of *Bromus auleticus* seed lots differing in *Neotyphodium* endophyte incidence: lots 5198 and 5216 E+ (100% seeds infected); lot 5211 E+/- (60% seeds infected) and lot 1037 and 1043 E- (0% seeds infected). Different letters indicate significant differences, $P < 0.05$, based on one-way ANOVA and Tukey post-hoc comparison.

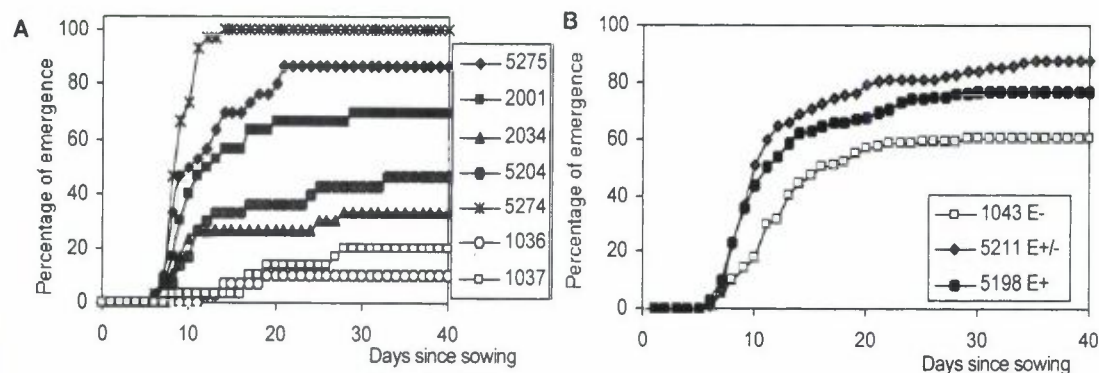


Figure 2. Percentage of seedling emergence at 23°C (A) or 29°C (B) in lots of *B. auleticus* differing in endophyte incidence: 80–100% (E+) (black symbols), 40–60% (E+/-) (grey symbols) or endophyte free (0%) (E-) (white symbols).

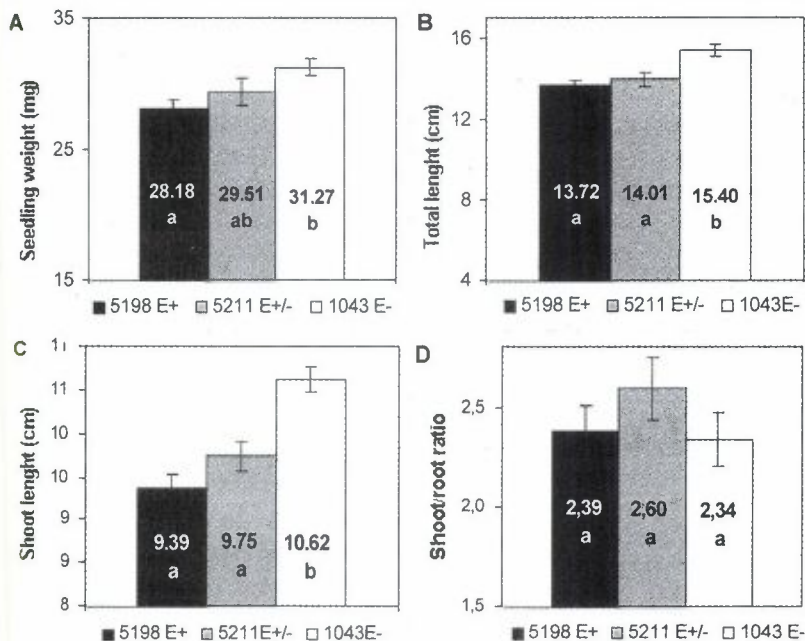


Figure 3. Ten-day-old seedling growth parameters in E- (0% plants infected), E+/- (60% of plants infected) and E+ (100% plants infected) *Bromus auleticus* lots. Means (\pm S.E.). A) fresh weight. B) total length. C) shoot length. D) shoot/root ratio. Different letters show significant differences $P < 0.05$, based on one-way ANOVA and Tukey post-hoc comparison.

Table 1. Accession numbers (Lot), endophyte infection level (%), final percentage, rate of emergence and emergence mean times of populations differing in endophyte infection level, studied in the emergence tests at 23°C and 29°C.

Temperature	Population	Endophytes incidence	Percentage of emergence (%)	Emergence rate (1/day)	Time (days)
23°C	1036	0	10 a	0.0667	15 a
	1037	0	20 a	0.0517	19 a
	2034	50	33.33 b	0.0813	12.30 ab
	5204	90	46.67 b	0.0696	14.36 ab
	2001	80	70	0.0847	11.81 b
	5274	100	100	0.1091	9.17 b
	5275	100	86.67	0.0878	11.38 b
29°C	1043	0	60.74 a	0.078	12.81 a
	5211	60	87.73 b	0.0813	12.3 a
	5198	100	77.06 b	0.0846	11.81 a

Values followed by different letters are significantly different at $P < 0.05$.

between 46.6–100% in the E+ lots, whereas PE reached 33% in the E+/- lots and almost 20% in the E- lots (Fig. 2A). Chi square test performed over the E- and the two E+ or E+/- lots displaying the lowest PE showed no significant differences between the E+ and the E+/- ($P=0.113$) lots or between both E- ($P=0.263$) lots. However, the PE in the E+ and E+/- lots was higher than that of the E- lots ($P<0.001$). Mean time of emergence ranged between 11.8 to 14.4 days for the E+ and E+/- lots and between 15 to 19.3 days for the E- lots ($P<0.01$). Tukey comparison showed that these differences were significant for three E+ lots whereas it showed no significant differences between the E+/-, one of the E+ and both E- lots (Table 1). Accordingly, the ER of the E+ and E+/- lots (which ranged from 0.07 d⁻¹ to 0.11 d⁻¹) was higher than the ER of the E- lots (which ranged from 0.05 to 0.07, approximately) (Table 1).

In the assay carried out at 29°C, seedling emergence started on the sixth day after sowing, for the three lots tested. Seedling emergence showed the same behaviour along the time for three lots (Fig. 2B). PE of the E- lot was significantly lower ($P<0.001$) than that of the E+ and E+/- lots; and no significant differences existed between the E+ and E+/- lots ($P=0.143$) (Table 1). The mean time of emergence (T) increased as endophyte incidence decreased, but the differences were not significant ($P>0.05$). The E+ lots exhibited the highest emergence rate, followed by the E+/- and E- lots (Table 1).

Seedling development

Ten-day-old E- seedlings were significantly heavier ($P=0.015$) than those from the E+ lots but not than those from the E+/- lots (Fig. 3A). These lots also exhibited both a higher total length (Fig. 3B) ($P<0.001$) and longer shoots (Fig. 3C) ($P<0.001$) than seedlings from the E+ and E+/- lots. Seedlings from the E+/- lots did not exhibit significant differences when compared with those from the E+ lot with regards to these variables. No significant differences were observed in the numbers of full-expanded leaves (two in all cases), root length (not shown) and shoot length/root length ratio among the three lots (Fig. 3D).

Plants from the E+ lots produced more tillers during one year of growth than those from the E- lot ($P<0.05$). The number of tillers produced by plants from the E+/- lot (which was only measured for six months and was not included in the statistical analysis) was intermediate when compared with the E+ and E- lots (Fig. 4).

Regrowth capacity

Leaf mass (Fig. 5A) produced after consecutive clippings decreased in both lots. Leaf mass produced by plants from the E- lots during the first regrowth period was significantly higher than that produced by the E+ ones. These differences were not significant ($P>0.05$) from the

second period onwards, and slightly reversed in the fourth period, as indicated by significant (clipping x level of endophyte infection in the lot) interaction and simple-effect analysis ($P<0.05$). E+ plants showed a greater capacity to regenerate the clipped biomass (RGR) (Fig. 5B). The length of the longest leaf decreased significantly with the clippings, but E+ plants presented longer leaves than E- plants after each regrowth period (Fig. 5C). The same behaviour was observed with regards to tiller numbers with the successive clippings, but this tendency was slightly more pronounced in E+ plants (Fig. 5D).

During the treatment, E- plants presented a mortality of 20%, whereas all E+ plants survived the treatment; however, this difference seemed to be marginally significant ($P=0.053$).

4. Discussion

This work allowed us for the first time a partial characterisation of the association between *B. auleticus*, a native grass from Argentinean pampas, and their *Neotyphodium* endophytes under controlled conditions. It is also one of the few works carried out on wild-native plants from natural populations (Saikkonen et al., 1999; Faeth, 2002; Pan and Clay, 2002; N... et al., 2003; Faeth et al., 2004).

Since seeds and plants used in our work belong to different populations, we have taken into account that part of the observed differences among the plants from the E+, E+/- and E- lots studied could be attributed to differences in genotypes. *Bromus auleticus* is a halogamous species, and the studied seed-plant lots were collected in populations that exist in a close proximity to each other. Thus, genetic flux

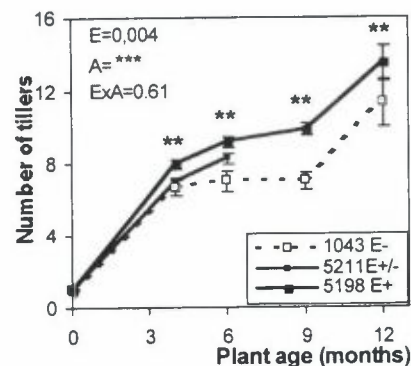


Figure 4. Number of tillers (mean (\pm S.E.)) produced by plants of *Bromus auleticus* from E+ (100% plant infected) and E- lots over one year, and over six months by a E+/- lot (60% plants infected). The P values for endophyte infection (E), plant age (A), and endophyte infection x plant age (ExA) obtained by repeated measures ANOVA are shown in the upper left of the panel. Asterisks indicate significant differences ($P<0.01$ (**), $P<0.001$ (***)).

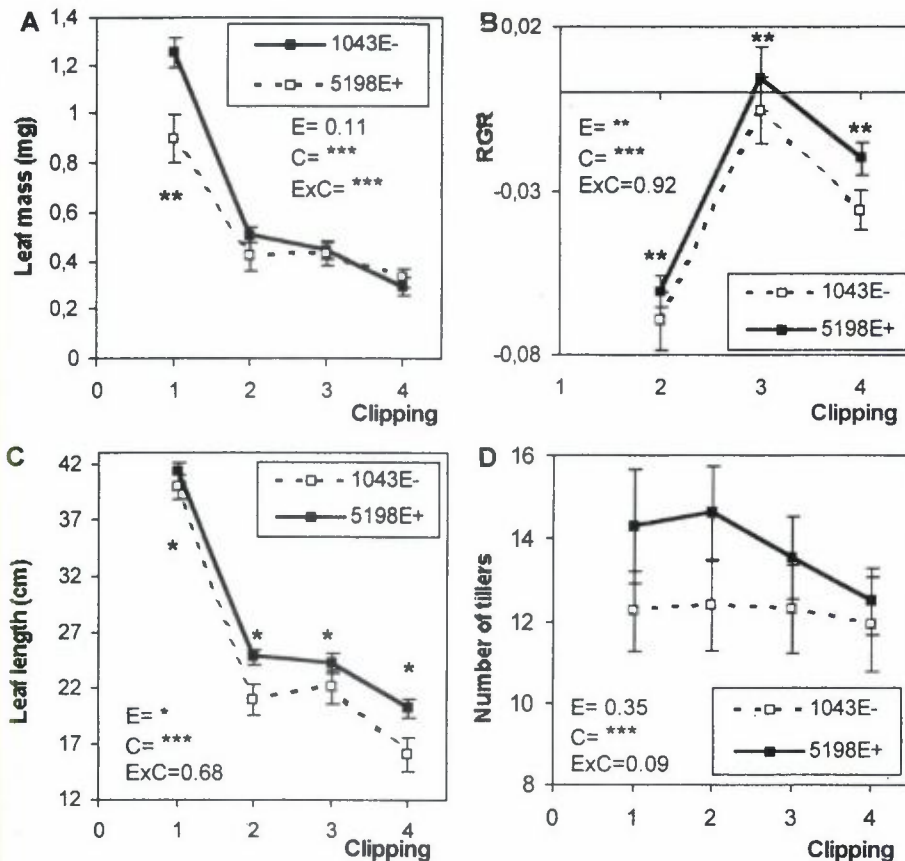


Figure 5. Regrowth capacity of plants from E+ and E- lots of *B. auleticus* during a resistance to defoliation experiment in which plants were clipped four times with a two weeks regrowth period between consecutive clippings. A) regenerated leaf dry matter (DM); B) regrowth rate (RGR); C) leaf length and D) number of living tillers, measured after each 14-day regrowth period. Values are means (\pm S.E.). The P values for endophyte infection level (E), regrowth period (clipping, C) and endophyte infection level \times regrowth period (ExC), based on repeated-measures ANOVA are shown in each panel. Asterisks indicate that means are significantly different ($P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***)).

might have occurred and we expect genetic differences to be minimal. Isozyme analyses reveal a high genetic similarity between populations of *B. auleticus* from Uruguay (more than 400 km from each other) and do not show a relationship between distance and genetic similarity of the populations (Dalagnol et al., 2001). On the other hand, intermediate values measured in plant characteristic from the E+/- lot may indicate an association between the incidence of endophytes in the lot and the parameters measured.

The costs and benefits of the grass-endophyte association depends on the level of resources (Ahlhom et al., 2002; Lehtonen et al., 2005). Thus, the results of this work should be considered under the controlled conditions of growth and fertilisation of this work. Another factor to take into account when analysing our results, is the fact that the loss of the endophyte, in this kind of symbiosis, is considered as a disorder in the plant (Lehtonen et al., 2005). E- plants used in our work were probably E+ and they lost the endophyte, which could affect their growth and responses to the different treatments.

In this study, seeds from the E+ lots were lighter than those from the E- ones, thus showing a negative association between seed weight and endophyte incidence. Agroclimatic conditions of the collection sites were very similar (De Fina, 1992), and should not have had a significant effect on the observed differences in seed weights.

In grasses without endophytes, seed weight is negatively correlated with germination time and positively correlated with germination percentage (Larsen and Andreasen, 2004). In spite of having lighter seeds, lots associated with endophytes completed the process of emergence in less time and showed higher emergence percentage and emergence rate than the E- lots. Thus in *B. auleticus*, these relationships appear to be modified by the presence of endophytes, thus favouring the lighter seeds of E+ plants.

Higher emergence percentage and rate seem to be a feature associated with endophyte presence, as observed in *F. arundinacea*, *L. perenne* and *B. setifolius* (Clay, 1987; Keogh and Lawrence, 1987; Joost, 1995; Novas et al.,

2003). In a previous study in Patagonia, seeds from E+ *B. setifolius* populations were heavier, and seedling emergence percentage and rate were higher than those from E- populations. The authors suggested that this behaviour could represent an advantage for E+ plants, since greater seed weight with greater reserve content could result in better seedling survival in poor nutrient soils and drought stressing conditions of Patagonia. Seed dispersion would not be affected by its weight because of the strong wind characteristic of this region (Novas et al., 2003).

In *B. auleticus*, the lighter weight of seeds from E+ populations could be advantageous for seed dispersion without affecting seedling survival in the Pampas, a humid region with mild winds and soils rich in nutrients. The lighter seeds, together with a higher percentage and faster seedling emergence, would allow a better dispersion and colonisation capacity by seeds from E+ populations (Lake and Leishman, 2004).

Ten-day-old plants from the E+ and E+/- lots were smaller than those from the E- ones. This may seem to be a disadvantage of the infection. However, it should be taken into account that seedlings from infected lots emerged between 1 to 5 days earlier than those from the uninfected lots. Thus, these differences could be compensated with time, because E+ plants will have a size corresponding to 11- to 15-day-old plants when the E- ones are 10 days old.

Plants from the E+ lots produced more tillers than those from the E- lots, a fact that indicates a high capacity of vegetative reproduction by E+ plants. Similar effects were observed in the clonal grass *Glyceria striata* infected with *Epichloë glyceriae*, where E+ plants allocated more of their total mass to clonal growth (Pan and Clay, 2003).

Regrowth capacity in perennial grasses depends on stored nutrients in remaining tissues as well as the allocation of nutrients for regrowth (Brown and Blazer, 1965; Boerener et al., 1990; Belesky and Fedders, 1996). In this study, successive clippings significantly affected plants of both lots. Plants from the E- lot produced more leaf mass than those from the E+ lots in the first regrowth period only. However, E+ plants exhibited longer leaves and higher productivity (RGR) in each regrowth period. In addition, plant mortality was higher in the E- lot. This could account for different strategies in order to obtain and use nutrients and photosynthates by E+ and E- plants. In E- plants, stored nutrients are used to produce heavier but shorter leaves, whereas in E+ plants, longer and lighter leaves are produced, a fact that could represent a lower energetic cost of regrowth and a greater capacity to intercept light. Thus E+ plants would have a high specific leaf area, a fact positively associated with a higher regrowth rate (Poorter and Remkes, 1990). Consecutive clippings could be compared to the effects of vertebrate herbivores on plants; therefore, observed characteristics would indicate an increased resistance to herbivore by E+ plants. As *B. auleticus* is not toxic to cattle, the endophyte would confer resistance to herbivory not by producing toxic compounds but by promoting the

regrowth of the plants. These advantageous properties observed in plants from E+ lot could account for the high incidence of endophytes observed in all natural populations of *B. auleticus* analysed across its distribution area in Argentina (data not shown), particularly because this area has been historically subjected to an intensive grazing by introduced cattle.

Our results seem to be partially congruent with those obtained in other studies performed on native grasses infected with *Neotyphodium* endophytes. Seedling emergence is enhanced in *B. setifolius* (Novas et al., 2003), but negatively affected in infected *F. arizonica* (Hamilton and Faeth, 2005).

Infected plants of *F. arizonica* produce fewer tillers and less biomass than infected ones (Faeth et al., 2004). In our work, endophyte infected plants exhibited an increased capacity of vegetative reproduction (tillers). As observed in the first regrowth period, E+ plants produced less leaf mass than E- plants. This would indicate a disadvantage of the endophyte infection. However, when stressful conditions were maintained over time (four defoliation events every two weeks) E+ plants outperformed the E- ones.

In *F. arizonica* E+ plants performed better than E- ones during long periods of drought, only (Morse et al., 2002; Faeth et al., 2004). Based on their results, the authors considered the relationship between *Festuca arizonica* and its endophyte as a parasitic one, but they proposed that endophytes favour E+ plants during long drought periods.

Our results in *B. auleticus*, under experimental conditions, and those obtained with *B. setifolius* under experimental (Novas et al., 2003) and natural conditions (White et al., 2001) would indicate that under the growth conditions of these works, the symbiosis between these two native grasses from Argentina and their endophytes would not be parasitic. In fact, these studies, together with those performed in native grasses from the North hemisphere, support the hypothesis that the relationship between wild-native grasses and endophytes is highly complex. These interactions range from pathogenic to mutualistic ones depending on grass species, endophyte, and environmental conditions (Saikkonen et al., 1998; Morse et al., 2002). Moreover, the higher resistance to periodic defoliation in E+ plants agrees with the hypothesis that endophytes would confer advantages to their hosts under stressful conditions maintained along time (Morse et al., 2002; Faeth et al., 2004).

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