

Beneficial effects of *Neotyphodium tembladerae* and *Neotyphodium pampeanum* on a wild forage grass

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Abstract

Asexual, vertically transmitted fungal endophytes of the genus *Neotyphodium* are considered to enhance growth, stress resistance and competitiveness of agronomic grasses, but have been suggested to have neutral or deleterious effects on wild grasses. We studied whether the associations between *Bromus auleticus* and their endophytes, *Neotyphodium pampeanum* and *Neotyphodium tembladerae*, are beneficial for this wild forage grass native to South America. In a greenhouse experiment, 3-month-old endophyte-infected plants (E+) showed enhanced growth relative to their endophyte-free counterparts (E–). In a one-year-long experiment in the field, E+ plants showed higher survival and regrowth rate, and produced more biomass and seeds than E– plants. Only with respect to the seed output did *N. tembladerae* seem to be detrimental, whereas *N. pampeanum* did not affect this parameter. No differences were observed with respect to the germination of seeds produced by E+ or E– plants. Loline alkaloids were detected in *N. pampeanum*-infected plants. Our results show that, similar to results with agronomic grasses, in wild grasses, the symbiosis with *Neotyphodium* species could be mutualistic.

Keywords: endophytes, wild grasses, *Bromus*, growth, reproduction

Introduction

Many cool-season grass species establish symbiotic associations with fungal endophytes of the genus

Epichloë Tul. and their asexual derivatives of the genus *Neotyphodium* A.E. Glenn, R.T. Hanlin et C.W. Bacon. *Neotyphodium* species are considered to be strictly vertically transmitted from the mother plants to the daughter plants via seed infection, without altering the reproduction of the host (Schardl *et al.*, 2004) (but also see Tadych *et al.*, 2007). Thus, the reproduction of the endophyte seems to depend on the reproductive success of the host plant.

The effects of asexual endophytes of grasses (*Neotyphodium* spp.) on plant growth and stress tolerance have been thoroughly studied in such forage grasses as *Lolium arundinaceum* (= *Schedonorus arundinaceus* = *Festuca arundinacea*), *Lolium multiflorum* and *Lolium perenne* (Cheplick and Faeth, 2009). In these host species, infected plants exhibit increased biomass production, regrowth capacity and tolerance to different stresses (Clay and Holah, 1999; Clay and Schardl, 2002; Schardl *et al.*, 2004; Malinowski and Belesky, 2006; Kuldau and Bacon, 2008). In addition, the endophytes protect their hosts from different herbivores by producing any of various alkaloids including lolines and peramine (active against herbivore insects) (reviewed in Bush *et al.*, 1997; Schardl *et al.*, 2004, 2007), as well as indole-diterpenes and ergot alkaloids that induce different disorders in mammalian herbivores (reviewed in Raisbeck *et al.*, 1991; Schardl *et al.*, 2006; Young *et al.*, 2006).

Because of the benefits that the endophyte confers to the infected plants and the high incidence of endophytes in naturalized populations of these species in the USA and New Zealand, these interactions are considered to be mutualistic. However, studies of endophyte effects on grass cultivars have been criticized because they may fail to capture the breadth of variability inherent in wild grass–endophyte populations and communities (Saikkonen, 2000; Saikkonen *et al.*, 2006).

In recent years, the number of studies conducted in order to evaluate the effects of the endophytes in native grasses has increased and the results seem to be variable

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and contradictory. In some cases, endophytes promote plant growth (Groppe *et al.*, 1999; Novas *et al.*, 2003; Iannone and Cabral, 2006), and in other cases, the endophytes have neutral or negative effects (Faeth *et al.*, 2010) or their effects depend on the host genotypes and abiotic conditions (Ahlholm *et al.*, 2002). Among the native grasses, the symbioses between *Festuca arizonica* and *Neotyphodium* spp. are the best studied. Whereas short-term greenhouse experiments indicate that endophytes may be detrimental for infected plants (Morse *et al.*, 2002; Faeth *et al.*, 2004; Faeth and Hamilton, 2006), long-term experiments conducted under field conditions have shown some beneficial effects of the endophytes on this host (Faeth, 2009).

In Argentina, *Neotyphodium tembladerae* D. Cabral et J.F. White seems to be the most common seed-borne grass endophyte, infecting more than ten grass species (Gentile *et al.*, 2005; Iannone *et al.*, 2009). Whereas some grasses are toxic to livestock because of their association with *N. tembladerae* (Cabral *et al.*, 1999), other grass species are not toxic in spite of possessing this endophyte species. Among the hosts of *N. tembladerae* that are non-toxic to cattle, *Bromus auleticus* is considered an excellent forage owing to its high productivity, high protein content and drought resistance (Moraes and Oliveira, 1990; Millot, 1999). This native grass inhabits different environments in grasslands and hills in the central region of Argentina, Uruguay and southern Brazil. Although in most regions *B. auleticus* possesses *N. tembladerae*, in grasslands of the Pampa region, it is instead infected with *Neotyphodium pampeanum* (Iannone *et al.*, 2009). In native *B. auleticus* populations in Argentina, the frequencies of infection with either *Neotyphodium* species are consistently near 100% (Iannone *et al.*, 2011a). Owing to its potential as forage, breeding programmes are being conducted in Argentina, Uruguay and Brazil in order to evaluate and improve the forage characteristics of this species (Millot, 2001). However, the presence of endophytes has not been considered in these programmes. A previous study in the greenhouse, performed with plants infected with *N. pampeanum*, indicated that endophyte-infected (E+) plants have a higher regrowth rate than E- plants (Iannone and Cabral, 2006).

The aim of this work was to characterize the effect of the vertically transmitted endophytes, *N. pampeanum* and *N. tembladerae*, on the growth of the native grass, *B. auleticus*, under greenhouse and field conditions.

Materials and methods

Plant material

Two populations of *Bromus auleticus* (Trin ex Nees) from different regions of Argentina, differing in their symbi-

otic *Neotyphodium* species, were studied. In the population, located in Intendente Alvear (35°15'S, 63°39'W), La Pampa province (henceforth designated 'La Pampa'), plants are infected with *N. pampeanum* (Iannone *et al.*, 2009). In the population from El Palmar National Park (31°53'S, 58°16'W) in Entre Rios Province (henceforth designated 'El Palmar'), the plants are infected with *N. tembladerae* (Iannone *et al.*, 2009). The percentage of infected plants in both populations is 100% (Iannone *et al.*, 2009, 2011a).

Propagation of endophyte-infected and endophyte-free seeds

In December 2004, seeds from thirty plants were collected in each of the above populations. Seeds from each population were pooled, and the presence of endophytes was checked by the inspection of fifty seeds of each pool. Seeds were softened in NaOH (10% v/v) for ten hours at room temperature and stained with aniline blue according to Clark *et al.* (1983). All the checked seeds were endophyte-infected (E+), and the seeds from each population were stored at room temperature.

After 8 months of storage, seeds were germinated in trays with perlite/commercial garden soil (1:1) in a growing chamber at 24°C with photoperiod 14/10 h light/dark. Two months after germination, when the seedlings had one or two tillers, they were screened for endophyte presence. Leaf-sheath segments of 1 cm were cut from the basal portion of the tiller (0.5 cm above the crown) and softened in NaOH for 15 min at room temperature. Segments were then rinsed with tap water and the parenchyma exposed by removing the adaxial epidermis. Plant tissues were then stained with aniline blue according to Clark *et al.* (1983). Endophyte presence was checked by inspection with an optical microscope. If necessary, all tillers were cut and checked to confirm that the plants were E-. Approximately 60% of the seedlings were endophyte-free, and E+ and E- seedlings were allowed to regrow in order to obtain E+ and E- seeds from these plants. To achieve this, in October, ten E+ and ten E- plants from each population were transplanted to 7 L pots and grown under field conditions, in the experimental field of the University of Buenos Aires. In January 2006, seeds of E+ and E- plants from each population were collected, pooled according to the endophytic status of the mother plant and stored at 5°C to be used in the experiments described below. It should be considered that these E+ and E- seeds were obtained from E+ and E- plants from different populations, grown in the same experimental field, thus cross-pollination among plants from both populations was possible and would give rise to seeds bearing maternal and paternal genotypes from

different populations. However, in plants from La Pampa, anthesis occurred 2 weeks before those from El Palmar, so the effects of cross-pollination among plants from different populations should be small.

Greenhouse studies of plant growth

In June 2006, E+ and E− seeds of each population were germinated in Petri dishes with filter paper moistened with distilled water. Nineteen E+ and 19 E− seedlings from El Palmar, and 77 E+ and 92 E− seedlings from La Pampa, were transplanted to 7 cm diameter × 12 cm deep pots filled with commercial soil. The seedlings were allowed to grow for 3 months (July, August and September) in the greenhouse of the EEA-INTA-Concepción del Uruguay (32°29'S and 58°20'W) in Entre Ríos province, Argentina. In early October (Spring), the height (in cm) of the seedlings and the number of tillers were measured and one tiller of each plant was cut to check the endophyte status as described above. All of the seedlings from E+ seeds were endophyte-infected and, as expected, no *Neotyphodium*-like hyphae were observed in seedlings obtained from E− seeds.

Field experiments

In early October 2006, 15 E+ and 15 E− plants from El Palmar, and 40 E+ and 40 E− plants from La Pampa, were removed from their pots and planted in the field of the EEA-INTA-Concepción del Uruguay. This site is located 560 km from Intendente Alvear (La Pampa) and 70 km from El Palmar National Park. Plants from La Pampa were planted in an arrangement of eight alternated E+ and E− rows (4 E+ and 4 E−) with ten plants per row. The distance between rows was 1 m, and the distance between plants in each row was 50 cm. Plants from El Palmar were planted in a plot of three rows with ten plants in each row as described above. In the central row, 5 E+ and 5 E− plants were planted. Weeds were removed before planting and thereafter removed by hand monthly during the experiment. Plants were only watered after transplanting, and no water other than rain was applied during the assay. The soils where the field experiment was performed are deficient in phosphorus; thus, to avoid effects of nutrient deficiency, fertilizer was applied only once in August 2007 (Winter), with $H_4(PO_4)_2Ca$ to provide 2 g P m^{−2} and urea at 5 g N m^{−2}.

During 2007, the plants were cut at the end of each season and allowed to regrow until the next season. Thus, cuts were applied on March 20th (end of Summer), June 17th (end of Autumn), September 14th (end of Winter) and December 27th (end of Spring) when seeds were mature and before the seeds shattered. The biomass produced by each plant during

each regrowth period was collected in paper bags. In December, all plants had produced culms that were counted and harvested separately by hand and stored in paper bags. Five seeds of each E+ plant were separated to check endophyte infection as described above, and all E+ plants produced E+ seeds.

Vegetative biomass produced in each regrowth period was oven-dried at 60°C to constant weight and then weighed to determine dry mass (DM). Culms with seeds were allowed to dry completely for 20 d at room temperature. Culms and seeds of each plant were weighed, and then seeds were separated by hand and weighed after removing unfilled seeds to assess the seed biomass produced by each plant. One gram of seeds of each plant was used to generate four pools of seeds: one pool for each population and endophytic status.

Measured variables in field growth experiments

Plant mortality in the field was measured during the year-long experiment.

Total above-ground biomass produced by each plant was calculated using the biomass values (as DM) produced by each plant during each growth season.

Relative growth rate (RGR) in each growing season was calculated as a logarithmic function of the ratio of DM produced in two consecutive regrowth periods divided by the number of days (d) in the growth interval: $RGR = \ln(DM_n/DM_{n-1})/d$ (Iannone and Cabral, 2006). As the biomass of the plant at the time when taken to the field was not established, we started measuring RGR in the Autumn using the biomass produced during October 2006 to 30 March 2007 as initial biomass.

Seed biomass of each plant was determined as explained above. The 1000 seed weights of E+ and E− plants of each population were determined for the seeds harvested in December 2007. Four replicates of 100 seeds of each plant were weighed and the average weight multiplied by 10.

The seed biomass and the 1000 seeds weight were then used to calculate the number of seeds produced by each plant using the formula: [(seed biomass × 1000)/1000 seeds weight].

To study differences among E+ and E− plants in resource allocation during the reproductive period, the seed output (Thompson and Stewart, 1981) was determined as the number of seeds divided by the biomass (DM) produced during spring (last regrowth period considered) (modified from Harper and Ogden, 1970).

Seed germination

With the pools of seeds generated in December 2007, we studied the effect of the endophytes on seed

germination (after 6 months storage at 5°C). Three replicates of 100 seeds of each population and endophyte status were germinated in Petri dishes with filter paper moistened with distilled water. The number of germinated seeds was recorded daily until no more seeds germinated. The final percentage of seed germination (G) and the mean germination time (T) were calculated as the average of each of the three replicates. The percentage of seeds germinated was calculated as $(G = \sum n / N)$, and the mean germination time was calculated as $(T = \sum tn / \sum n)$, (where t is the number of days since sowing, n the number of germinating seeds this day and N the number of sown seeds), as indicated in Peretti (1994) and Bewley and Black (1994).

Statistical analysis

Owing to the number of seedlings obtained for greenhouse experiments and differential plant mortality in the field between E+ and E- plants in both populations, one-way unbalanced ANOVA was used to study differences in all of the measured variables between E+ and E- seedlings and plants of each population.

Two-way ANOVA, with endophyte and population as fixed effects, was performed to compare the effects of the endophytes between populations on the measured variables. To accomplish the assumptions of the model, datasets were balanced with fourteen or thirteen plants per population and endophytic status. Data were *log*- or *sin*-transformed when necessary.

Comparisons of plant mortality between E+ and E- plants of each population during the field assay were performed using Fisher's exact test for the El Palmar population and chi-square test for the La Pampa population.

Differences in relative growth rate (RGR) during the experiment were evaluated with repeated measures ANOVA; in this case, datasets were balanced to 13 E+ and 13 E- plants for the El Palmar population and 30 E+ and 30 E- plants for the La Pampa population. The

Kruskal–Wallis test and one-way unbalanced ANOVA were used to test differences in final percentage of seed germination and in the mean germination time, respectively.

Loline production analyses

Lolines were analysed as in Blankenship *et al.* (2001). Seeds obtained from E+ and E- plants from the field experiments in December 2007 were germinated, planted in pots and grown in a greenhouse for 3 months. Ten E+ and three E- plants from each population were analysed. Plants were cut, ground with liquid N₂ and lyophilized. Alkaloids were extracted from 0.1 g of freeze-dried plant material with 100 µL of 1 M NaOH and 1 mL of chloroform in 2-mL tubes; tubes were shaken for 1 h at room temperature. Quinoline was used as internal standard, and lolines were determined using gas chromatography–mass spectrometry.

Results

Seedling growth

After 3 months of growth in the greenhouse, E+ plants from both populations were significantly taller than E- plants ($F_{1,38} = 12.8$; $P = 0.0009$, for El Palmar and $F_{1,168} = 11.1$; $P = 0.001$, for La Pampa) (Figure 1a).

Furthermore, E+ and E- plants from La Pampa were significantly taller than those from El Palmar (two-way ANOVA, $F_{1,52} = 8.39$; $P = 0.006$).

No significant differences ($P > 0.05$) were observed in the number of tillers between E+ and E- plants from El Palmar; however, E+ plants from La Pampa produced more tillers than E- ones (one-way ANOVA, $F_{1,168} = 10.1$; $P = 0.0018$) (Figure 1b).

The differences observed between plants from La Pampa and El Palmar with respect to the endophyte effect on the number of tillers were also reflected by the significant endophyte \times population interaction

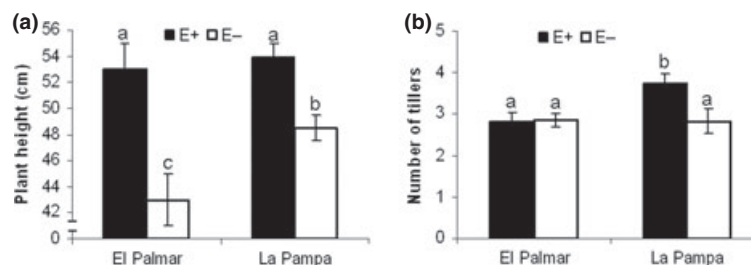


Figure 1 Height and tiller number in 3-month-old plants, endophyte-infected (E+) or uninfected (E-), from two populations of *Bromus auleticus* grown in a greenhouse. In the El Palmar population, plants are associated with *Neotyphodium tembladerae*, and in the La Pampa population, plants are associated with *N. pampeanum*. In each panel, different letters indicate significant differences ($P < 0.05$).

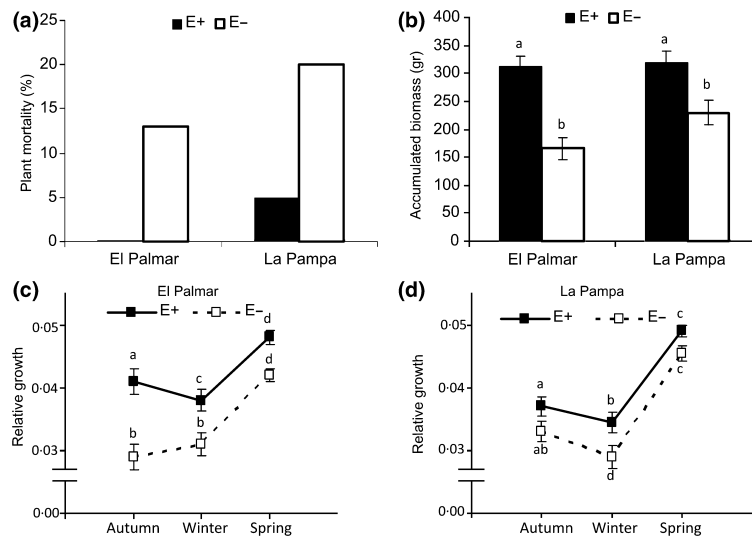


Figure 2 Plant mortality and growth of *Bromus auleticus* from two different populations during a 1-year-long assay conducted in the field. The El Palmar population is associated with *Neotyphodium tembladerae* and the La Pampa population is associated with *N. pampeanum*. (a) Plant mortality, (b) Accumulated above-ground dry biomass produced in the field from October 2006 (early Spring) to December 2007 (early Summer) and (c) and (d) relative growth rate in each season. In each panel, different letters indicate significant differences ($P < 0.05$).

($F_{1,52} = 4.07$; $P = 0.046$). Simple effects analyses showed (once again) significant differences only between E+ and E- plants from La Pampa ($F_{1,52} = 4.1$; $P = 0.04$).

Field experiments

Plant survival

The 15 E+ plants (100%) from El Palmar survived through the entire experiment, but two out of 15 E- plants (13%) died during the Summer before the first cut was applied, but this difference in survival was not significant (Fisher's exact test $df = 1$; $P = 0.24$) (Figure 2a). On the other hand, the mortality of E- plants from La Pampa (eight out of 40, 20%) was significantly higher than E+ plants (two out of 40, 5%) ($df = 1$, $P = 0.036$) (Figure 2a).

Growth and total biomass

Above-ground biomass amounts produced by E+ plants of both populations in each regrowth period were larger than that produced by the E- ones (data not shown). These differences were reflected in total above-ground biomass produced during the first year of growth (Figure 2b). Endophyte-infected plants from El Palmar produced twice the biomass of E- ones ($F_{1,26} = 18.1$; $P < 0.001$), whereas in plants from La

Pampa, the E+ ones produced approximately 30% more biomass than E- ones ($F_{1,67} = 14.9$; $P = 0.0002$), indicating a significantly greater effect of the endophyte on plants from El Palmar than those from La Pampa (endophyte \times population interaction $F_{1,48} = 4.8$; $P = 0.031$).

In both populations, significant differences were also found in RGR (Figure 2c and d) both among the regrowth periods ($F_{2,48} = 65.5$; $P < 0.0001$ in El Palmar and $F_{2,116} = 237$; $P < 0.0001$ in La Pampa) and between E+ and E- plants of both populations ($F_{1,24} = 27.2$; $P < 0.0001$ in El Palmar and $F_{1,58} = 13.6$; $P < 0.0001$ in La Pampa). For E+ plants from El Palmar (Figure 2c), RGR was higher than the RGR of E- plants; however, whereas E+ plants showed a decrease in the RGR in Winter, the RGR of E- plants increased in each successive growing period (significant interaction between time and endophyte status, $F_{2,48} = 7.7$; $P = 0.001$) (Figure 2c). Simple effects analyses showed that RGR of E+ plants was significantly higher than RGR of E- plants in Autumn and Winter ($F_{1,48} = 23.7$; $P < 0.0001$ and $F_{1,48} = 6.1$; $P = 0.01$, respectively) and marginally significant in Spring ($F_{1,48} = 3.1$; $P = 0.09$). The RGR of E+ or E- plants from La Pampa showed similar relationships, (Figure 2d). Repeated measures ANOVA showed that RGR of E+ plants was significantly higher during Winter ($F_{1,58} = 23.6$; $P < 0.0001$) and marginally higher in Autumn and Spring ($F_{1,58} = 3.1$; $P = 0.08$ and $F_{1,58} = 3.7$; $P = 0.06$, respectively).

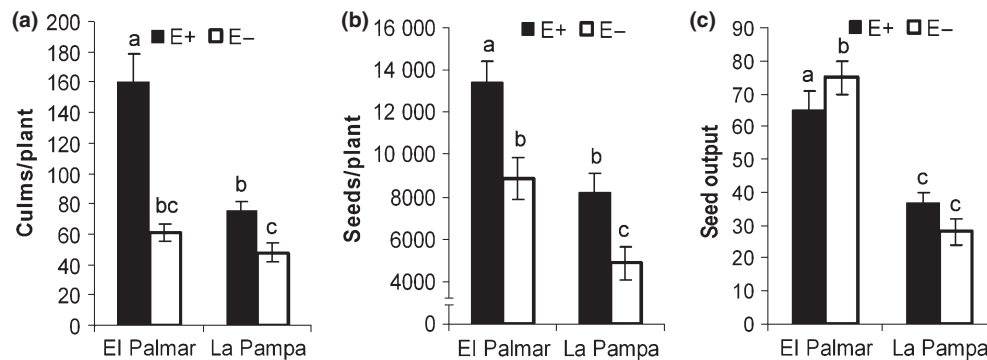


Figure 3 Reproductive characteristics of E+ and E- plants of *Bromus auleticus* from two different populations, El Palmar population infected with *Neotyphodium tembladerae* and La Pampa infected with *N. pampeanum*. (a) number of culms per plant, (b) number of seeds per plant, (c) Seed output, calculated as the number of seeds divided by the total dry biomass produced in the Spring period. In each panel, different letters indicate significant differences ($P < 0.05$).

Reproductive characters

Endophyte-infected plants from both populations produced more culms than E- plants ($F_{1,26} = 7.2$; $P = 0.001$ for El Palmar and $F_{1,53} = 14.5$; $P = 0.0004$ for La Pampa). Plants from El Palmar produced more culms than plants from La Pampa. ($F_{1,48} = 13.7$; $P < 0.0006$) (Figure 3a).

No differences were observed in the 1000-seed weights between E+ and E- plants of each population ($P > 0.05$). However, 1000 seeds from La Pampa (5.23 g for E+ and 5.06 g for E-) were greater than those from El Palmar (4.56 g for the E+ and 4.68 g for E-) ($F_{1,8} = 48.8$; $P = 0.0001$).

Endophyte-infected plants of both population produced more seeds than E- ones ($F_{1,48} = 28.1$; $P < 0.0001$) (Figure 3b), although no differences were observed with respect to the number of seeds per culm between E+ and E- plants within each population (data not shown).

The seed output in E- plants from El Palmar was significantly higher than in E+ plants ($F_{1,48} = 4.3$; $P = 0.04$), although no differences were detected between E+ and E- plants from La Pampa (Figure 3c).

Seed germination

No significant differences were observed in the final germination percentages of seeds produced by E+ or E- plants of both populations ($H = 7.07$, $P = 0.70$). The final germination percentages were 93 and 94% for seeds produced by E+ and E- plants, respectively, from El Palmar and 94% for seeds produced by both E+ and E- plants from La Pampa. The mean germination time was 10.1 and 10.6 d for seeds produced by E+ and E- plants from El Palmar, respectively, and 9.8 and 10.1 d for seeds produced by E+ and E- plants from La Pampa,

respectively. These differences were non-significant ($F_{1,374} = 2.37$, $P = 0.0698$).

Loline alkaloid detection

Loline alkaloids were only detected in endophyte-infected plants from La Pampa (infected with *N. pampeanum*). All E+ plants from La Pampa produced the same pattern of loline alkaloid production because loline, *N*-methylloline, *N*-acetylnorloline and *N*-formylloline were detected. However, no *N*-acetylloline was detected in these samples. The total amount of lolines in leaves was in the range of 250–2500 $\mu\text{g g}^{-1}$ DM.

Discussion

In this study, we found that *N. pampeanum* and *N. tembladerae* promote plant growth in two populations of the wild native grass, *B. auleticus*, under field experimental conditions. Endophyte-infected plants generally exhibited enhanced seedling growth and survival in the field. Positive effects of the endophytes were also detected in growth and seed production by adult plants. However, no effects of the endophyte on seed germination were detected and some negative effects were detected on seed output only in El Palmar population. In addition, we found high loline alkaloid levels in plants infected with *N. pampeanum*, but none in plants with *N. tembladerae*.

The benefits of epichloid endophytes on plant performance have been mainly studied in agronomic grasses. However, it is known that the effects of the endophyte on growth outcomes are associated with plant and endophyte genotypes (Cheplick *et al.*, 2000; Hesse *et al.*, 2003; Morse *et al.*, 2007), and with adaptations of the grass–fungus symbiota to local environments or to the conditions of the experiments (Sullivan

and Faeth, 2008). In addition, most of the studies on wild native grasses indicate that the endophytes have variable effects on seed germination, seedling growth, plant survival, growth and reproduction, ranging from pathogenesis to mutualism (Clay and Schardl, 2002; Faeth *et al.*, 2004; Wäli *et al.*, 2009). However, in native grasses from South America, results presented here and in previous studies (Novas *et al.*, 2003; Iannone and Cabral, 2006) indicate that vertically transmitted endophytes have significant positive effects in most of the plant characteristics studied.

In this work, we did not focus on each single plant genotype; *Bromus auleticus* is an outcrossing species (Rivas, 2001; Gutiérrez *et al.*, 2006), and high intra-population genotypic variability exists in each population (Moraes and Oliveira, 1990; Dalagnol *et al.*, 2001). Our experiments were carried out with only two populations, so given the genotypic variability of this host, our results should not be extrapolated to other populations of this grass species. However, we consider that the number of individuals per endophytic status studied in each population (15 and 40) are adequate to represent the genotypic variability present in each population and are similar to those used for similar comparisons by other authors (De Battista *et al.*, 1990; Zabalgoeazcoa *et al.*, 2006; Swarthout *et al.*, 2009).

In agreement with observations in other endophyte-grass associations (Cheplick *et al.*, 2000; Hesse *et al.*, 2003), in our work, interactions between plant and endophyte genotypes (at the population level) were detected in some of the traits studied, because endophytes had different effects on plants from different populations. Whereas *N. pampeanum* had a positive or neutral effect on most of the variables studied on plants from La Pampa, *N. tembladerae* had no effect on tiller number in 3-month-old plants and on plant establishment in the field, and a negative effect on seed output of plants from El Palmar.

Endophyte-infected 3-month-old plants from both populations were taller than E- plants, and E+ plants from LaPampa produced more tillers than E- plants. These differences in the initial growth could represent the advantage of E+ seedlings.

In both populations, endophyte-infected plants exhibited a higher RGR in most of the growth periods. This feature would indicate that the higher biomass produced by E+ plants during each regrowth period would be due to a more efficient use of the resources by E+ plants and not to differences in the biomass produced before being transplanted in the field or during the Spring after being transplanted. This result is also consistent with those obtained in the greenhouse with plants infected with *N. pampeanum* (Iannone and Cabral, 2006), where E+ plants showed higher regrowth rate than E- plants.

The higher number of culms (reproductive tillers) and seeds produced by E+ plants could be associated with their higher production of biomass. However, in plants from El Palmar, the higher seed output observed in E- plants indicates that E- plants allocated more resources in seed production than E+ plants. Notwithstanding this, and considering that E+ plants produced more seeds than E- plants and that no differences were observed between the germination of E+ and E- seeds of both populations, E+ plants should produce more descendants than E- plants.

The detection of lolines in the *B. auleticus*-*N. pampeanum* symbiotum, and not in the *B. auleticus*-*N. tembladerae* symbiotum, is consistent with the detection by PCR of the *lolC* gene in *N. pampeanum* isolates (Iannone *et al.*, 2011b). This result indicates that E+ plants from La Pampa could be better protected against herbivorous insects, because lolines have anti-insect activity and have been shown to protect against insects in other symbiota of grasses and *Neotyphodium* species (Siegel *et al.*, 1990; Riedell *et al.*, 1991; Wilkinson *et al.*, 2000; Gonthier *et al.*, 2008). However, new experiments are needed in order to assess this possibility.

Bromus auleticus inhabits a region that in the last 300 years has been subjected to a high pressure of grazing by livestock and currently is found as small populations scattered in its distribution area in places protected from the livestock. The higher regrowth rate of the E+ plants observed in this and in a previous study (Iannone and Cabral, 2006) could represent an advantage for E+ plants subject to grazing pressure. Thus, our results, and the high incidence of endophytes in populations of this host, suggest that the endophytes may have played a fundamental role in the adaptation and survival of this species under grazing pressure.

Our results are informative with regard to the use of the endophytes in *B. auleticus* breeding programmes. Peramine has been reported in *N. tembladerae*-infected plants of *Bromus setifolius* (White *et al.*, 2001), and diagnostic PCR indicates that the *Neotyphodium* strains found in *B. auleticus* would not produce ergot alkaloids, but *N. tembladerae*-infected plants could produce some indole-diterpene (Iannone *et al.*, 2010). Although toxicity to cattle has not been reported for this species, more studies should be conducted in order to characterize the alkaloid profiles and potential toxicity of symbiota host species.

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