

Imperfect Vertical Transmission of the Endophyte *Neotyphodium* in Exotic Grasses in Grasslands of the Flooding Pampa

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Abstract Cool-season grasses establish symbioses with vertically transmitted *Neotyphodium* endophytes widespread in nature. The frequency of endophyte-infected plants in closed populations (i.e., without migrations) depends on both the differential fitness between infected and non-infected plants, and the endophyte-transmission efficiency. Most studies have been focused on the first mechanism ignoring the second. Infection frequency and endophyte transmission from vegetative tissues to seeds were surveyed in two grasses growing in vegetation units that differ in flood and grazing regimes, and soil salinity. Transmission efficiency and infection frequency for tall fescue did not vary significantly and were 0.98 and 1.00, respectively. For Italian ryegrass, transmission efficiency and infection frequency were 0.88 and 0.57 in humid prairies, and 0.96 and 0.96 in the other vegetation units. Only in humid mesophytic meadows, the observed pattern was irrespective of the presence or absence of grazers. Our results suggest that selection forces for endophyte

infection are different for both species. Imperfect transmission was only compensated in tall fescue through an increased fitness of infected plants. Interpreting variations of infection frequency only in terms of differential fitness can be misleading, considering that endophyte transmission can be imperfect and variable in nature. Therefore, this study highlights the importance of measuring transmission efficiency.

Introduction

Animals and plants usually host symbiotic microorganisms from which they obtain novel metabolic capacities [15–17] and may ultimately determine host evolution and invasion ability [38, 39, 48]. Ecologists have been interested, for a long time, in the processes that control symbioses persistence, especially focusing in host fitness and transmission mode [9, 10, 12, 16]. Symbiosis between animals and gut bacteria, plants and soil bacteria or fungal mycorrhiza, and plant–pathogens are among the most-studied interactions [9, 15, 29, 40] and recently, the widespread symbiosis between cool-season grasses (Poaceae) and *Neotyphodium* endophytes (Ascomycetes: Clavicipitaceae) is becoming an important case of study [9, 40, 47]. *Neotyphodium* is mainly vertically transmitted via host seeds; they sparsely sporulate (or even not at all), and are not transported by pollen [32, 44]. The symbiosis between *Neotyphodium* endophytes and grasses is frequently considered as a defensive mutualism [8]. The fungi receive nutrition, protection and dispersion from the host plant while the plants may benefit from endophyte-induced changes in phenotypic traits that increase resistance to biotic and abiotic stresses [9, 31]. In particular, several fungal toxic

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alkaloids accumulate in host-plant tissues conferring resistance to vertebrate and invertebrate herbivores [9, 10]. Nonetheless, this symbiosis is sometimes regarded as parasitic because under certain growth condition, some metabolic costs in host plants have been observed [7, 17, 18].

The frequency of endophyte infection (i.e., the proportion of endophyte-infected plants in grass populations) can vary in time and space, ranging from 0 to 100% of infected plants [4, 10, 19, 43, 48]. In many studies, the frequency of infection has been used as a measure of fitness enhancement of endophyte-infected vs. non-infected plants. Thus, in an attempt to understand the natural forces selecting for this symbiosis, the endophyte-infection frequency of the populations has been correlated to specific environmental factors, such as livestock grazing [21, 26], insect herbivory [10], drought [30], herbicide exposure [48], altitudinal gradient [4, 19], and different attributes of the vegetation community [30, 39]. A positive association between endophyte-infection frequency and a given environmental factor has been used to infer the positive effect of the endophyte on host fitness [4, 19, 28, 34]. At the same time, the lower endophyte-infection frequency found in extremely harsh or poor environments has been considered as the cost in fitness that the endophyte infection implies for host plants (e.g., [4, 19, 28, 34]).

However, the relationship between endophyte-infection frequency and environmental variables is not always direct. In the absence of migrations among different local populations (see [42]), two mechanisms may account for endophyte-infection frequency: the differential fitness between endophyte-infected and non-infected plants and the endophyte vertical transmission efficiency (i.e., the proportion of infected seeds produced by infected plants; [8, 20, 37, 42]). The rate at which the frequency of endophyte infection approaches equilibrium (i.e., 1.00) will change in direct relationship with the magnitude of host fitness enhancement only if endophyte vertical transmission is 1.00 (i.e., perfect transmission) [8]. Prior works have suggested that *Neotyphodium* spp. have highly efficient vertical transmission via seeds produced on the infected plants [9, 11]. However, a few experimental results indicate that failures in endophyte transmission may exist at different plant phenological stages [1, 5]. These inefficiencies can result from the lack of endophyte ability to re-infect young seedlings, to colonize tillers, spikes or panicles, spikelets and ovaries during flowering, or to die before seed germination [1, 5, 23, 49]. Unlike to other symbiosis such as “plant–pathogen” and “aphids–gut bacteria” [12, 29], there is not much information on variations in the endophyte–transmission efficiency for the *Neotyphodium* endophyte–grass symbiosis under natural conditions [1, 5, 11].

In this work, we present results from a survey of *Neotyphodium* infection frequency and transmission effi-

ciency from vegetative plant tissues to seeds in tall fescue (*Schedonorus arundinaceus* Schreb., Dumort (=/*Festuca arundinacea*/Schreb.) [45], and Italian ryegrass (*Lolium multiflorum* Lam.) populations from grasslands of the Flooding Pampa, Argentina [46]. The existence of variation in the endophyte–transmission efficiency and frequency will improve our understanding on controls of endophyte–infection dynamics and persistence in grass populations. These European grasses were introduced as high-quality forage to improve winter production, and today, have successfully colonized the major vegetation units in the Flooding Pampa [46]. Prior surveys have indicated that they are the only species in the current flora of the Flooding Pampa showing high *Neotyphodium* endophyte–infection frequencies [14]. While perennial tall fescue plants are usually toxic for cattle because of the alkaloids produced by *N. coenophialum* [9], there is no record of cattle intoxication foraging annual Italian ryegrass plants infected with *N. occultans* [14]. We examined *Neotyphodium* spp. presence in seeds collected from plants growing in vegetation units which differ in flood and grazing regimes, and soil salinity [6, 24, 36]. This spatial heterogeneity in vegetation structure and function was used to survey the natural variability in the efficiency of endophyte vertical transmission.

Methods

Study Area

The Flooding Pampa is a flat and poorly drained area that covers 90,000 km² between 35° and 38° S latitude in east-central Argentina [46]. The climate is sub-humid temperate, with mean monthly temperatures ranging from 7°C in winter to 22°C in summer. Mean annual precipitation is 990 mm, distributed throughout the year with small peaks in autumn and spring. Flooding events are frequent in lower areas, generally, from autumn through late spring, while the higher areas are much affected by the summer droughts. Flooding and drought represent stressful constraints to plant productivity and species composition [24]. Cattle breeding is the main productive activity in the area, and differences in grazing management impose drastic changes in the plant community’s composition [6].

Perelman et al. [36] described four grassland types that differ in soil chemistry, plant composition and primary productivity (for more details see references [2] and [6]). A major variation in plant species composition across these grasslands was observed at very fine spatial scales (0.1–10 km²), due to subtle topographic differences and soil salinity gradients [36]. ‘Mesophytic meadows’ appear in the highest positive areas where soils are deep, well-drained, acidic, and non-saline, being the most productive

grassland type. ‘Humid mesophytic meadows’ cover flat areas at intermediate topographic positions; soils are characterized by an acidic and non-saline A1 horizon but a saline and highly alkaline B2 horizon. ‘Humid prairies’ cover extended lowlands frequently subjected to flooding with a saline and highly alkaline horizon close to the soil surface. ‘Halophytic steppes’ cover small depressions in flat areas; soils are very shallow and alkaline with high salt content in the upper layer, determining their low productivity.

Collection of Plant Seeds and Endophyte Determination

Plants bearing seeds were sampled at the end of the 2004 growing season from at least four stands (sites) of each of the four grassland types described above. The sampled stands were separated by more than 0.7 km and were selected within an area of 25.80 km² representing the landscape heterogeneity of the Flooding Pampa. In humid mesophytic meadows we also sampled four ungrazed stands (exclosures) yielding a total of five vegetation units for this study. The exclosures, that have more than 4 ha, were established in different years and, at present, have been without grazing for over 20 years.

Mature seeds were harvested from three to five spikes per plant of tall fescue and Italian ryegrass plants collected along a linear transect within each stand. In order to avoid sampling the same plant twice, the distance between harvested plants was greater than 2 m. Seeds from each individual plant (a total of 408) were separated at the laboratory and air dried. Tall fescue was found in only one stand of both the ‘humid prairies’ and the ‘halophytic steppes’ (Table 1) and since we did not find enough seeds, these vegetation units were not considered when analyzing this species results.

The presence of the *Neotyphodium* endophyte was assessed using light microscopy, as visual examination in mature seeds has been found to be an accurate method [4, 27, 41]. The seeds present the greatest amount of hyphae

per unit of biomass reducing the likelihood of false negative or positive [41, 44]. In addition, *Neotyphodium* species infecting *S. arundinaceus* (*N. coenophialum*) and *L. multiflorum* (*N. occultans*) have been morphologically well-described [32, 47], allowing to identify and to recognize each species from other seed fungi without growing them in plate culture.

Seeds were incubated for 12 h in sodium hydroxide (2.5%), and later stained with Rose Bengal (ethanol 5 ml, Rose Bengal stain 0.5 g, distilled water 95 ml; [3]). A plant was considered infected if at least one of ten examined seeds was infected. If the ten seeds were negative, another 30 seeds per plant were checked for endophyte presence. A plant was considered non-infected when all its seeds were endophyte-negative. Endophyte-infection frequency was expressed as the proportion of endophyte-infected plants within each vegetation unit. The endophyte-transmission efficiency was calculated only on infected plants, as the number of infected seeds divided by the number of evaluated seeds. The endophyte transmission from one generation to the next involves the passage of hyphae through a series of subsequent stages that contain seed to seedling, infected seedlings to adult plant vegetative tissues (tillers) and, finally, vegetative tissues to seeds [8, 37, 42]. Therefore, the presence of an infected seed necessarily means that the previous steps have been fulfilled in the same surveyed plant. For this reason, transmission efficiency measured as the proportion of infected seeds integrates such processes, and it is an estimation of plant to seed transmission. We are aware that the visual examination technique does not discriminate between alive from dead endophytes. For this reason, we could be overestimating the endophyte-transmission efficiency.

Characterization of the Vegetation Units

Previous studies provide an adequate description of local grassland type attributes and soil characteristics (see

Table 1 Structural and functional attributes for each vegetation unit during 2004

	Vegetation cover (%) ^a			NDVI ^b			
	Total	Italian ryegrass	Tall fescue	Autumn	Winter	Spring	Summer
Mesophytic meadows—Grazed	95.0(1.9)a	11.7(5.8)a	25.0(8.8)ab	0.69(0.01)a	0.52(0.03)a	0.64(0.03)a	0.71(0.02)a
Humid mesophytic meadows—Grazed	96.7(1.0)a	11.1(4.8)a	22.2(13.0)ab	0.67(0.02)a	0.47(0.02)ab	0.52(0.02)bc	0.66(0.02)b
Humid mesophytic meadows—Ungrazed	92.9(2.8)a	3.0(1.8)a	52.9(12.7)b	n.d.	n.d.	n.d.	n.d.
Humid prairies—Grazed	91.0(3.1)a	11.0(4.7)a	0.4(0.2)a	0.66(0.01)a	0.50(0.02)a	0.58(0.02)ac	0.68(0.01)c
Halophytic steppes—Grazed	84.4(4.7)a	1.8(1.6)a	5.7(3.7)a	0.63(0.02)a	0.40(0.01)b	0.46(0.01)c	0.63(0.02)d

^a Average values of vegetation cover estimated by a modified Braun–Blanquet scale [33]. Standard errors (in brackets; $n=4$ stands) indicate cover spatial variability within vegetation units. Letters indicate significant differences ($p<0.05$).

^b NDVI (Normalized Difference Vegetation Index) values correspond to the seasonal average per vegetation unit during the 2004 growing season. Standard errors (in brackets; $n=4$ stands) indicate NDVI spatial variability within vegetation units. Letters indicate significant differences ($p<0.01$). n.d. no data available.

synthesis by [6] and [36]). However, a complementary structural and functional study of the grassland stands was carried out in 2004 during the plant sampling period. This detailed information could help in finding local factors related with infection frequency and transmission efficiency variation found in different community stands. Total vegetation cover and percentage cover of tall fescue and Italian ryegrass were visually estimated in four stands of each of the five vegetation units using a modified Braun–Blanquet scale [33]. In addition, we used the Normalized Difference Vegetation Index (NDVI) to functionally characterize each vegetation unit. NDVI is a spectral index computed from red and infrared bands that is directly related to the fraction of absorbed photosynthetically active radiation (fAPAR). This index can therefore be used as an estimate of aboveground net primary productivity (ANPP). We used NDVI data provided by MODIS/Terra images (MODIS/Terra Vegetation Indexes 16-Day L3 Global 250 m, version V004) with NDVI values corresponding to the seasonal average per vegetation unit during the 2004 growing season. MODIS images have a spatial resolution of 250 m×250 m (pixel size) and high temporal resolution. The methodology has proved to be accurate differentiating vegetation units in this system (for more details see reference [2]).

Statistical Analyses

Data of the frequency of endophyte-infection and transmission efficiency were strictly bounded, showed non constant variance, and errors were not normally distributed. Thus, minimum adequate models were estimated under Generalized Linear Models (Statistical Software R, package “glm”). Tests for the significance of the effects in the models were performed via the Wald statistic. We first assessed the environmental effect on *Neotyphodium* endophyte infection (i.e., binary response for each plant: infected or non-infected) in tall fescue and Italian ryegrass plants. For Italian ryegrass, models included binomial error distribution, logit link function, and tested the effects of one factor with five levels (each vegetation unit): the four grassland types under grazing and the ungrazed humid mesophytic meadows. We could not perform a similar analysis for tall fescue as there was no variability in the infection of the 109 surveyed plants (all plants were infected).

We performed two analyses to test for differences in transmission efficiency between species across the different environments. The first analysis included two factors, the species (two levels) and the vegetation unit (three levels: mesophytic meadows, humid mesophytic meadows and ungrazed humid mesophytic meadows). Since tall fescue did not have enough seeds either in the humid prairies or in the halophytic steppes, the second analysis included only

Italian ryegrass and was performed to evaluate differences in transmission efficiency across all five vegetation units (i.e., one factor with five levels). Models included ordinal multinomial distribution and logit link function. For each plant, transmission efficiency was classified into one of four classes. The first class included plants with less than or equal to 0.6 transmission efficiencies, which were represented in 3.7% of the sampled plants; the second class included plants with transmissions higher than 0.6, but lower than 0.9 and represented 4.2% of the sampled population; the third class included plants with transmissions higher than 0.9, but lower than 1 (10.5% of the plants). The fourth class was composed only of plants with 1.0 (perfect) transmission efficiency and was represented in 81.6% of the plants.

Total cover and individual species cover were analyzed by ANOVAs (i.e., one factor with five levels) to test for differences among vegetation units (Statistical Software R, package “lm”). Data was arc-sine transformed ($\sin^{-1} \sqrt{0.01 \times p}$ where p is percentage cover) to comply with ANOVA assumptions (normality and homoscedasticity). Tukey’s test was used for a posteriori comparisons. To evaluate differences in canopy dynamics among vegetation units, MODIS NDVI values for the different seasons were analyzed by repeated-measures ANOVA with two factors: vegetation unit and season (repeated measure).

Results

All the tall fescue plants were infected by *Neotyphodium* endophytes (1.0), regardless of the grazing condition or the grassland type where the host plants were growing (Fig. 1). Conversely, for Italian ryegrass, there were always non-infected plants in all the populations examined (Fig. 2). Furthermore, there were differences in the endophyte-infection frequency among populations of Italian ryegrass associated to different vegetation units. Whereas the humid prairies had a frequency of 0.57 of infected plants, the other vegetation units had 0.96 (Wald=37.4, $p<0.001$). Likewise, grazing had no effect on the infection frequency of this species (Fig. 2; Wald=0.1, $p=0.778$).

Endophyte transmission from vegetative plant tissues to seeds was, in general, very efficient for both species. Perfect transmission (=100% of seeds produced were infected) was observed in 311 of the 381 infected plants sampled. Moreover, transmission efficiencies in tall fescue and Italian ryegrass plants showed no significant differences either when growing in the humid meadows or in the humid mesophytic meadows, and were statistically equal regardless of the grazing condition (Figs. 1 and 2; species: Wald=0.04, $p=0.841$; vegetation unit: Wald=3.0, $p=0.227$; species×vegetation unit: Wald=3.5, $p=0.172$). Tall fescue

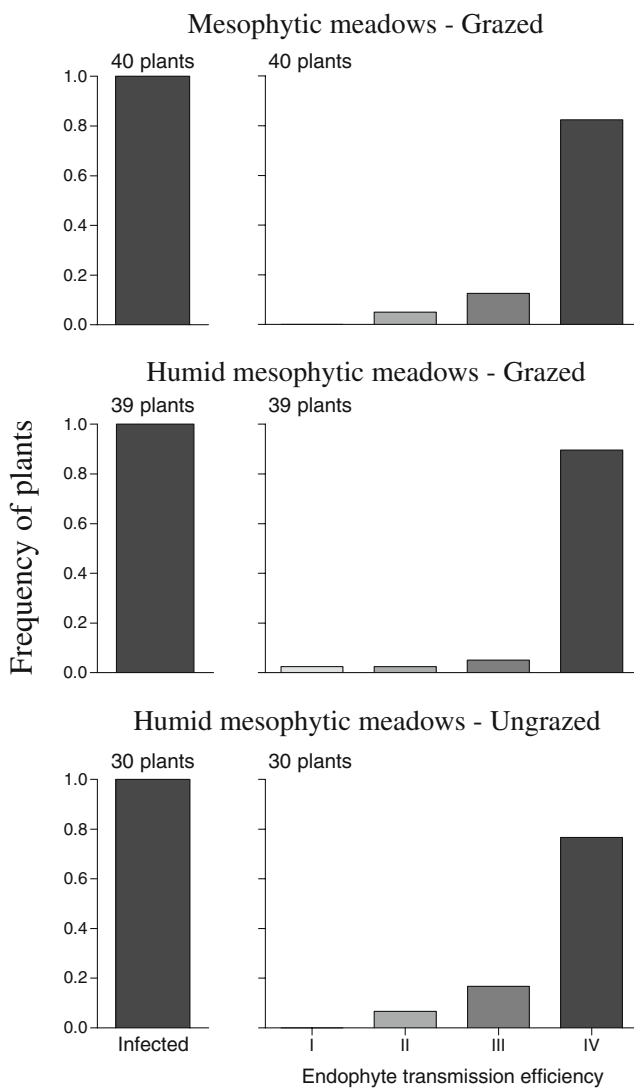


Figure 1 *Neotyphodium* endophyte-infection frequency (left panel) and endophyte-transmission efficiency (right panel) in populations of *Schedonorus arundinaceus* in different vegetation units in the Flooding Pampa grassland (Argentina). Grazed and ungrazed stands are only included for the humid mesophytic meadows. Upper left of each graph total number of observed plants (for infection frequency) or number of infected plants (for transmission efficiency) is given. I, II, III and IV represent classes of plant endophyte-transmission efficiencies (less than or equal to 0.6; higher than 0.6 but lower than 0.9; higher than 0.9, but lower than 1 and with 1 of transmission efficiency respectively). There were not differences among vegetation units in both variables

presented, on average, an endophyte transmission efficiency of 0.98. In Italian ryegrass, transmission efficiency for each plant was on average 0.88 in humid prairies and 0.96 in the other vegetation units (Fig. 2; Wald=5.1, $p=0.024$).

Total plant cover was nearly 100% in all vegetation units. Italian ryegrass cover was always lower than 12% (Table 1). Tall fescue cover was higher in ungrazed humid mesophytic meadows stands than in those of either grazed humid prairies or the halophytic steppes (Table 1). Italian

ryegrass cover was highly variable and particularly low in both ungrazed humid mesophytic meadows and the grazed halophytic steppes. However, average cover values were statistically equal (Table 1). NDVI showed a seasonal trend, with the lowest values in winter and the highest in summer (season: $F_{3,45}=148$, $p<0.01$; Table 1). This seasonal trend differed among vegetation units (vegetation unit: $F_{3,15}=11.23$, $p<0.01$; season \times vegetation unit: $F_{9,45}=2.55$, $p=0.01$). The differences among vegetation units were more evident in spring, when the mesophytic meadows showed NDVI values at least 10% greater than the rest of the vegetation units. The mesophytic meadows had the highest annual NDVI, while the halophytic steppes presented the lowest values.

Discussion

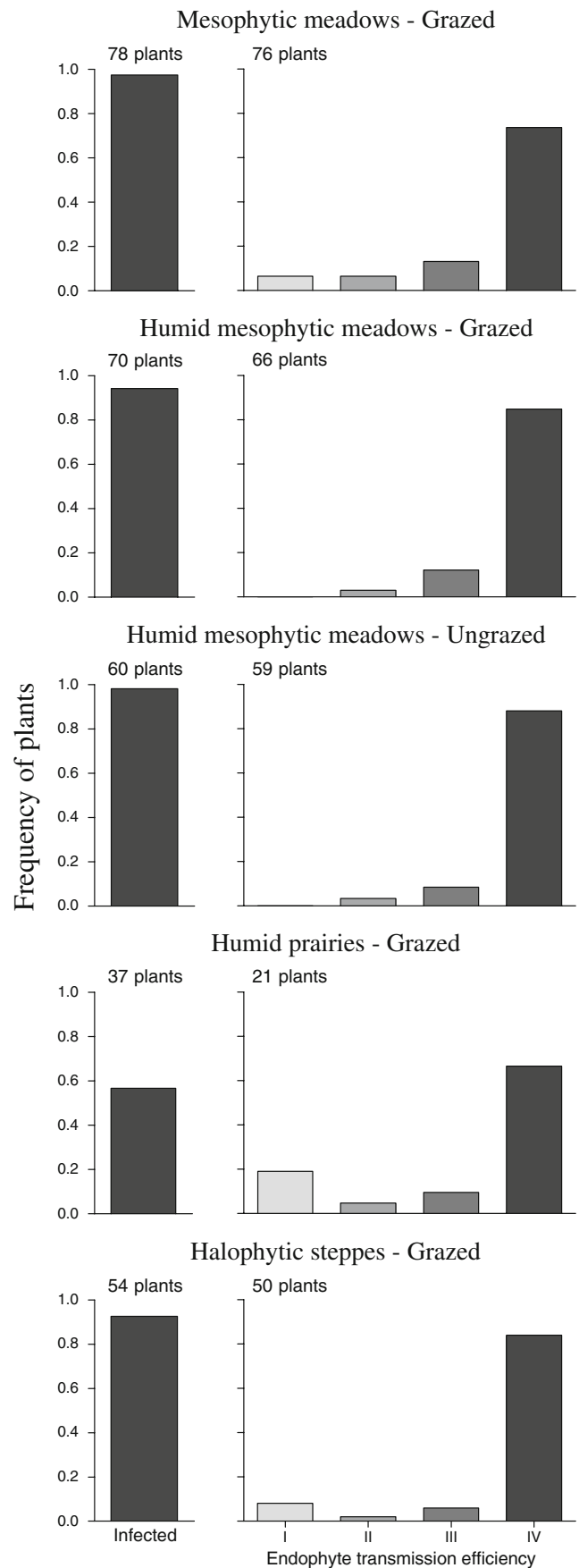
Neotyphodium vertical transmission from one generation to the next was imperfect for tall fescue and Italian ryegrass plants across major vegetation units in the Flooding Pampa. We did not find differences in transmission efficiency from vegetative tissues to seeds between species but we found differences in endophyte-infection frequency. These results suggest that selection forces for endophyte infection are stronger on tall fescue than on Italian ryegrass in those habitats where both species were present. Despite the production of non-infected seeds in all vegetation units, infection frequency for tall fescue was constantly 1.00. Conversely, Italian ryegrass populations always had a variable proportion of non-infected established plants, reinforcing the idea that imperfect vertical transmission can modify endophyte-infection frequencies in grass populations [20, 37]. Imperfect transmission impact on infection frequency determination is lower in perennial hosts than in annuals due to reseeding cycles. Therefore, as theory predicts for microbes exclusively vertically transmitted [12, 15, 17, 20, 22, 42], the persistence of a facultative symbiosis depends not only on the outcome of the inter-specific interaction, but also on the effectiveness of the endophyte-transmission mode [12, 20, 29].

Endophyte-transmission efficiency from plant to seeds may be important in maintaining non-infected plants, at least for Italian ryegrass, in Flooding Pampa grasslands. This result is in accordance with the recent reported cases on North American wild species [1] and on *L. rigidum*, a relative of *L. multiflorum* species [5], where the imperfect vertical transmission occurred depending on the population species. The endophyte-infection frequency has been used as a measure of the fitness enhancement of endophyte-infected vs. non-infected plants [21, 26, 30, 31, 48] without considering the contribution of the level of endophyte transmission. For instance, the positive correlation between

Figure 2 *Neotyphodium* endophyte-infection frequency (left panel) and endophyte-transmission efficiency (right panel) in populations of *Lolium multiflorum* in different vegetation units in the Flooding Pampa grassland (Argentina). Grazed and ungrazed stands are only included for the humid mesophytic meadows. Upper left of each graph total number of observed plants (for infection frequency) or number of infected plants (for transmission efficiency) is given. Endophyte-transmission efficiency classes as in Fig. 1. Humid prairies presented lower infection frequency and transmission efficiency than the other vegetation units

endophyte-infection frequency and drier climatic conditions in Europe has been interpreted as evidence for the higher fitness of infected plant over non-infected ones [30, 31]. However, we demonstrated that vertical transmission can be imperfect and can also vary among vegetation units. Endophyte-transmission efficiency in Italian ryegrass plants growing in humid prairies was 0.57 whereas the percentage of established infected plants was 0.57. Preservation of *L. multiflorum* seeds and their endophyte happens on the soil surface during spring and summer and germination waves occur during late fall and early winter [20]. Lowlands are usually subjected to flooding events [24]; and this condition may have negative effects on endophyte viability within the seeds [49]. Thus, the observed pattern for the endophyte-infection frequency and climatic conditions (at local and regional scales) may be, in fact, accounted for variations in the transmission of the endophyte rather than variations in the relative fitness [20]. Nonetheless, this lower endophyte-infection frequency, which could be caused by the lower vertical transmission, does not imply that endophyte-mediated fitness advantages are lower in this vegetation unit. Evidences suggest that the advantages of endophyte-infected over non-infected Italian ryegrass plants [35, 48] may not be enough to compensate for the imperfect vertical transmission. According to the model by Gundel et al. [20] the fitness ratio between non-infected and infected Italian ryegrass plants should be lower than 0.8 to compensate for 0.12 of inefficiency in endophyte transmission. Symbiosis will become extinct in local population in a few generations if there is no difference in fitness between non-infected and infected plants (ratio=1) [20, but see 42].

We observed no significant association between cattle grazing level and endophyte-infection frequency within populations of both exotic species in humid mesophytic meadows stands, despite that previous studies indicated that herbivores selective consumption or grazing pressure could result in variations in population infection frequency (e.g., [10, 21, 26, 28]). The low relative abundance of tall fescue and Italian ryegrass in these Pampean grasslands (see also [6] and [36]) could be diluting their possible toxic effects, which is in accordance with the low frequency of intoxicated animals reported in the area [14]. Alternatively, improved persistence and competitiveness of infected plants through endophyte-induced changes, not directly



related to herbivory resistance, could be the main reason for a high endophyte-infection frequency [17, 21, 31, 35, 48]. On the other hand, we did not observe an effect of cattle presence on the efficiency of endophyte transmission for both species. If cattle avoid tall fescue plants due to their toxicity, it is reasonable to expect no differences in endophyte-transmission efficiency between plants from grazed and ungrazed stands. But Italian ryegrass plants are heavily grazed due to their high forage quality and non-toxic biomass [14]; hence, our results suggest that endophyte-transmission efficiency would not have been affected by defoliation. Nonetheless, results from manipulative experiments are needed in order to understand the controls of endophyte vertical transmission at individual plant level. It is possible that populations with low endophyte-infection frequency result from individuals genetically differentiated that are poor hosts or have differences in their capability to produce endophyte-infected seed. Recent studies and our own results show that individual populations with low infection frequency have individuals with low transmission efficiency [1, 5] suggesting that endophyte vertical transmission could be a specific trait related to ecotypes from each vegetation community rather than a consequence of environmental differences. However, none of these studies have adequately separated genetic and environmental effects on these populations using for example common garden or reciprocal transplant experiments.

The survey did not allow the detection of potential mechanisms beneath such a pattern, since variations in endophyte-infection frequency and endophyte-transmission efficiency were not related neither to differences in the cover of the host-plant species, to total plant cover, nor to seasonal NDVI dynamics of the vegetation units. Other environmental factors (i.e., flooding, drought, salinity, heavy metals, and others) could be acting as drivers of differential fitness between infected and non-infected plants [9, 31, 48]. However, the spread of these exotic grasses through native communities agrees with many studies, which suggest that endophytes can increase host invasion ability [10, 39]. This could be especially important in tall fescue, where plant establishment seems to be highly dependent on endophyte infection, given that we found tall fescue seeds without endophytes, but we did not find any established plant free of endophyte. Endophyte infection could depress host fitness under certain conditions [7, 17], but indirect effects could also be claimed for the patterns we observed. Humid prairies and halophytic steppes are characterized by frequent flooding that often generates anaerobic and toxic soil environments and oxidative plant stress [24, 25]. These conditions can cause problems for endophyte growth within the plant affecting its transmission and in turn, by reducing the endophyte's herbivory protection, fescue seedling persistence. The small popula-

tions of established tall fescue seedlings, without the endophyte's protection could become a highly grazed resource and pushed to extinction [10]. Ultimately, this could explain the absence of the species in these vegetation units. However, local persistence of the endophyte could be sustained by migrations of endophyte-infected seeds from surrounding areas where endophytes have positive effects on host-plant fitness [42]. On one hand, absence of fescue seedlings would suggest that migrations are infrequent, but on the other, ryegrass migrations might occur and explain their presence in the unfavorable conditions of the lower topographic sites. Nevertheless, the contribution of this process might be low considering the presence of Italian ryegrass ecotypes at relatively short distance [13] and the low seed dispersion rate of grasses [50], unless another factor is acting as a seed vector (e.g., floods or grazing) without affecting endophyte survival (but see [44]).

In conclusion, this work shows that endophyte-transmission efficiency may depend on the vegetation unit and the grass-endophyte association considered, regardless of grazing condition although, for this factor, only the vegetation unit "humid mesophytic meadow" was assessed. Here, we showed that variations in endophyte-transmission efficiency can be found in nature, as was previously suspected [37, 42]. Thus, even if endophyte-infection frequency is related to endophyte fitness enhancement, the imperfect transmission could be altering the success of this symbiotic relationship [20, 37]. Several studies suggest that endophytes can improve fitness under stress conditions [26, 31] but ignore that transmission may have a large impact on the percent of infected plants. Future manipulative experiments should be carried out to determine the combination of interspecific interactions and abiotic factors that control the endophyte enhancement of host-plant fitness, the endophyte-transmission efficiency at different plant phenological stages, and the magnitude and direction of migration at different spatial and temporal scales.

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