



ENHANCEMENT OF PERFORMANCE IN TALL FESCUE BY ENDOPHYTE PRESENCE

MEJORA DEL RENDIMIENTO DE LA FESTUCA ALTA POR LA PRESENCIA DE ENDÓFITOS

Lucas R. Petigrosso^{1,2*}  & Osvaldo R. Vignolio¹ 

1. Facultad de Ciencias Agrarias,
Universidad Nacional de Mar del
Plata, Balcarce, Argentina

2. Facultad de Ingeniería y Ciencias
Agrarias, Pontificia Universidad
Católica Argentina (UCA), Ciudad
Autónoma de Buenos Aires.,
Argentina

*lpetigrosso@mdp.edu.ar,
lucaspetegrosso@uca.edu.ar

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SUMMARY

Background and aims: The invasion of exotic plant species can be facilitated by the presence of endophytes. Plants and caryopses of *Lolium arundinaceum* (tall fescue) are frequently infected by the endophytic fungus *Epichloë coenophiala*, which is toxic to livestock. This study evaluated the effect of *E. coenophiala* on vegetative and reproductive growth and caryopsis germination in tall fescue under defoliation, as well as fungal transmission from maternal plants to caryopses.

M&M: The experiment was conducted outdoors. Plants from two infection levels (infected and uninfected) were grown individually in pots. During the vegetative stage, shoot biomass was removed at 6 cm height every 230 °C days.

Results: All plants survived and reached the reproduction stage. Endophyte presence did not impose a cost on the evaluated traits. The relative growth rate of shoot biomass and the length of primary seminal root were significantly higher in infected than in uninfected plants. Infected plants tended to have higher reproductive effort, caryopsis number, and vegetative biomass per plant than uninfected plants. Defoliation did not disrupt vertical transmission of the endophyte to caryopses.

Conclusions: The results suggest that tall fescue plants infected with *E. coenophiala* may exhibit higher fitness than uninfected plants. These traits are likely to contribute to the successful propagation of tall fescue across different environments in the Pampas region.

KEY WORDS

Exotic, fitness, fungus, grass, grassland, *Lolium arundinaceum*, propagation.

RESUMEN

Introducción y objetivos: La invasión de especies vegetales exóticas puede verse facilitada por la presencia de endófitos. Las plantas y las cariopsis de *Lolium arundinaceum* (festuca alta) están frecuentemente infectadas por el hongo endófito *Epichloë coenophiala*, que es tóxico para el ganado. En este estudio se evaluó el efecto de *E. coenophiala* sobre el crecimiento vegetativo y reproductivo y la germinación de las cariopsis en festuca alta bajo condiciones de defoliación, así como la transmisión del hongo desde las plantas maternas hacia las cariopsis.

M&M: El experimento se realizó al aire libre. Se cultivaron individualmente en macetas plantas con dos niveles de infección (infectadas y no infectadas). Durante la etapa vegetativa, la biomasa aérea se removió a una altura de 6 cm cada 230 °C días.

Resultados: Todas las plantas sobrevivieron y alcanzaron la etapa reproductiva. La presencia del endófito no implicó un costo en los caracteres evaluados. La tasa de crecimiento relativo de la biomasa aérea y la longitud de la raíz seminal primaria fueron significativamente mayores en las plantas infectadas que en las no infectadas. Las plantas infectadas tendieron a presentar mayor esfuerzo reproductivo, número de cariopsis y biomasa vegetativa por planta que las no infectadas. La defoliación no interrumpió la transmisión vertical del endófito a las cariopsis.

Conclusiones: Los resultados sugieren que las plantas de festuca alta infectadas con *E. coenophiala* podrían presentar una mayor aptitud biológica que las plantas no infectadas. Estos caracteres probablemente contribuyan a la propagación exitosa de la festuca alta en diferentes ambientes de la región Pampeana.

PALABRAS CLAVE

Aptitud, exótica, gramínea, hongo, *Lolium arundinaceum*, pastizal, propagación.

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INTRODUCTION

The invasion of exotic plant species may be facilitated by the presence of endophytes (Bard *et al.*, 2024). This is the case of *Lolium arundinaceum* (Schreb.) Darbysh. [syn. *Festuca arundinacea* Schreb., *Schedonorus arundinaceus* Dumort, S. J. Darbyshire], commonly known as tall fescue, a perennial cool-season C₃ grass native to North Africa and Europe (Gibson & Newman, 2001). Tall fescue spreads primarily through caryopses, acts as an invasive species in grasslands across several countries, including the United States (Saikkonen *et al.*, 2016), Australia, New Zealand (Young *et al.*, 2013), and Argentina (Gibson & Newman, 2001). Tall fescue plants and caryopses are commonly infected by the asexual endophytic fungus *Epichloë coenophiala* (Morgan-Jones & W. Gams) C. W. Bacon & Schardl (Clay & Schardl, 2002; Iannone *et al.*, 2012; Leuchtmann *et al.*, 2014). The grass-fungus association establishes a mutualistic symbiosis with vertical transmission from maternal plants to caryopses (Clay & Schardl, 2002; Iannone *et al.*, 2012). The plant supplies the fungus with nutrients, soluble carbohydrates and a pathway for dissemination through the caryopses (Clay & Schardl, 2002; Cheplick, 2007). In turn, the plant benefits under biotic conditions are: i) defense against herbivores, through the production of alkaloids (ergovaline), which are toxic to cattle, sheep and horses (Decunta *et al.*, 2021; Realini *et al.*, 2024); ii) enhancement of germination, competitive ability, vegetative and reproductive growth (Malinowski & Belesky, 2000; Novas *et al.*, 2003; Vila-Aiub *et al.*, 2005; Baynes *et al.*, 2012; Iannone *et al.*, 2012; Moore *et al.*, 2019; Petigrosso *et al.*, 2020, 2021a); and under abiotic conditions are: iii) drought tolerance (Decunta *et al.*, 2021); nutrient poor conditions (Cheplick *et al.*, 1989) and flooding conditions (Saedi *et al.*, 2021; Petigrosso *et al.*, 2023, 2024, 2026a,b).

Due to its high agronomic importance as a temperate forage species, tall fescue was intentionally introduced in Argentina in the 1950s (Scheneiter *et al.*, 2015). Populations of infected tall fescue are widely distributed across grasslands, pastures, abandoned agricultural lands, and rural roads in the Pampas region of Argentina (Petigrosso *et al.*, 2013; Scheneiter *et al.*, 2015). Economic losses associated with livestock intoxication have

been estimated at approximately USD 54 million annually in Argentina (De Battista, 2005). The spread of infected tall fescue plants may be associated with increased fitness, through enhanced vegetative and reproductive performance compared to uninfected plants (Iannone *et al.*, 2012; Saikkonen *et al.*, 2016). This assumption is supported by studies conducted in the Flooding Pampa grasslands of Argentina, where the successful spread of the exotic species *Lolium multiflorum* infected with *Epichloë occulta* (formerly referred to as *Neotyphodium occulta*; Leuchtmann *et al.*, 2014) has been associated with enhanced vegetative and reproductive performance, as well as increased seedling recruitment (Vila-Aiub *et al.*, 2005; Uchitel *et al.*, 2011; Casas *et al.*, 2016).

In grasslands and pastures, tall fescue is managed under grazing conditions. In these ecosystems, defoliation represents a major selective force affecting plant growth, reproduction, and resource allocation (Ferraro & Oesterheld, 2002). The immediate effects of defoliation are a reduction in photosynthetic leaf area, stored carbohydrates, and shoot and root plant growth (Ferraro & Oesterheld, 2002). Under these conditions, maintaining endophytic survival and growth may incur additional costs in host plants on vegetative and reproductive growth (Petigrosso *et al.*, 2021a; García Parisi *et al.*, 2012; Ueno *et al.*, 2024). For example, in infected tall fescue plants, lower vegetative and reproductive growth during the recovery period after defoliation has been documented compared to uninfected plants (Petigrosso *et al.*, 2021b). Comparable results were reported by García Parisi *et al.* (2012), who studied the effects of defoliation on *L. multiflorum* plants infected by the endophyte *E. occulta*. The cost was associated with a reduction in plant growth and failures in the transmission of endophyte from the plant to the caryopses (García Parisi *et al.*, 2012).

Given the economic impact of infected tall fescue, there is a considerable interest in understanding the factors that influence the propagation in the Pampas region. A better understanding of the potential effects of the endophyte on tall fescue propagation can be achieved through analysis of the defoliation effects on vegetative and reproductive growth and fungus transmission.

The objective of this study was to evaluate, under defoliation conditions, the effect of *E. coenophiala*

on vegetative and reproductive plant growth and caryopsis germination. Fungal transmission from maternal plants to caryopses was also assessed.

MATERIAL AND METHODS

Study site and treatments

The experiment was conducted outdoors at Unidad Integrada Balcarce (EEA-INTA Balcarce, Facultad de Ciencias Agrarias – UNMDP, Buenos Aires, Argentina). Tall fescue plants were obtained from a naturalized wild population, whose caryopses infected with the endophyte *E. coenophiala* (E+) were collected from a pasture (Mar Chiquita County, 37° 32' S, 57° 55' W; Buenos Aires Province). Endophyte-free caryopses (E-) were obtained by storing E+ caryopses for 12 months at 20 °C and 52 % relative humidity (Petigrosso *et al.*, 2023, 2024). Endophyte presence was determined using the diagnostic technique described by Saha *et al.* (1988). As the endophyte was not detected in seedlings from a sample of 50 caryopses, the treatment was considered fully effective (Fig. 1).

On 31 July 2023, caryopses of tall fescue E+ and E- were germinated separately in a grow chamber and ten days later were transferred to seed trays. On 21 September 2023, one plant per infection level was transferred to plastic pots (16 cm diameter x 15 cm height) with drainage holes. The pots were filled with agricultural soil, classified as a Typical Argiudol, P, Bray method: 147.52 ppm; M.O., combustion method: 5.82 %; C: 3.37 %; Nitrate, Phenol Disulphonic method: 21.11 ppm, and pH, ratio 1:2.5, soil: water method: 8.90.

Sixty-two pots were used (31 E+ and 31 E-), and each one was placed into a plastic tray (22 cm long x 20 cm wide x 4 cm deep). The pots were periodically rotated, and the plants were watered with tap water as required. After six months of acclimation, on 29 February 2024, all plants were clipped at 6 cm above ground level to standardize the height; this biomass was not included in subsequent analyses (Fig. 2).

Vegetative growth

The experimental period began on 20 March 2024. Initial plant biomass was characterized

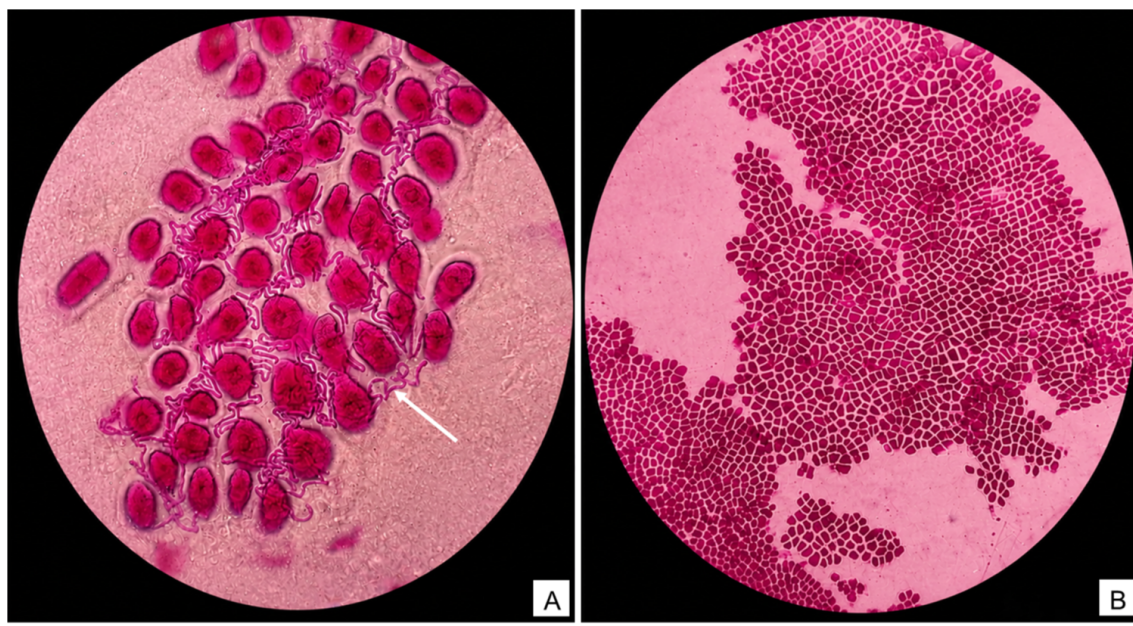


Fig. 1. A: Tall fescue caryopsis infected with the endophytic *Epichloë coenophiala*. The arrow indicates the convoluted hyphae of the endophytic fungus between the aleurone-bearing cells of the endosperm. **B:** Tall fescue caryopsis uninfected by the endophytic fungus. Scale bars= 50 µm.

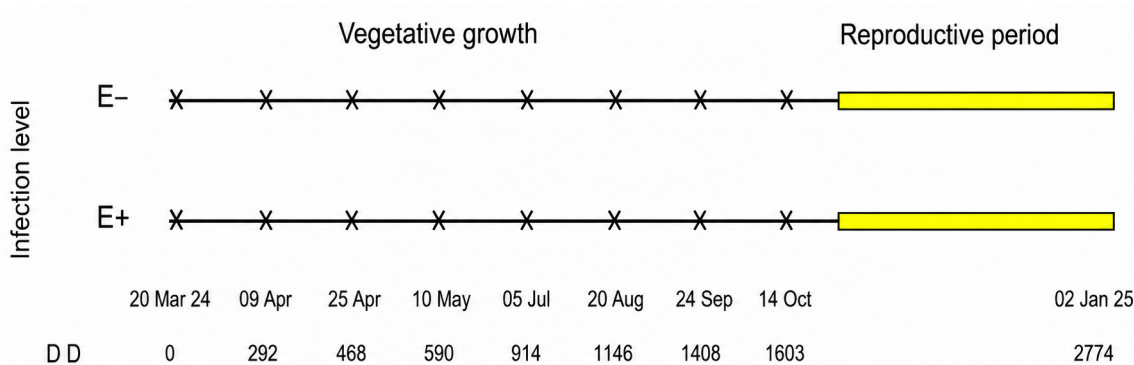


Fig. 2. Conceptual diagram illustrating the experiment conducted during the year 2024. References= E+ and E-: plant infected by *Epichloë coenophiala* endophyte and uninfected, respectively; X: shoot biomass harvest; D D: accumulated degree days.

through destructive sampling. Five plants per infection level were randomly chosen, and tiller number, above-ground and root biomass were determined. The root biomass was washed with tap water using a 1 mm sieve, and the soil was removed. Shoot and root biomass were placed in paper bags with their respective identification, dried in an oven at 60 °C for 96 h, and then weighed. The remaining plants, 26 per infection level, were subjected to defoliation during the vegetative stage, before internode elongation. Seven cuts were applied at a height of 6 cm: 20 March, 9 April, 25 April, 10 May, 5 July, 20 August, and 24 September 2024 (Fig. 2). The infected and uninfected plants were identified with numbers from 1 to 26. Defoliation was applied approximately every 230 °C days. This frequency is lower than that reported as high defoliation intensity (Petigrosso *et al.*, 2020). A defoliation frequency each 400 °C days is consistent with the foliar half-life period for the tall fescue (Insua *et al.*, 2012). A brief recovery period between cuts was applied in order to condition the plants' responses (Ferraro & Oosterheld, 2002).

Degree days (D.D.) were calculated using the following equation:

$$\sum D.D. = [(T \text{ max} + T \text{ min})/2] - T_b]$$

where T max is the maximum daily air temperature (°C); T min is the minimum daily air temperature (°C), and T_b is the base temperature of 4 °C for tall fescue (Petigrosso *et al.*, 2026a). Temperature data were obtained from EEA INTA Balcarce (Fig. 3). Global daily radiation data were provided by Dr. Dossio (pers. comm.).

The relative growth rate (RGR) was calculated as an indicator of plant resilience; that is, the plant's capacity to regrow and to regenerate its structure and function after a disturbance (defoliation) or a period of stress (Mooney & Hobbs, 1986):

$$RGR = (\ln w_2 - \ln w_1)/(t_2 - t_1)$$

where w₁ and w₂ are the dry weights of the aerial vegetative biomass per plant for each infection level, for times t₁ and t₂, respectively. The harvest at t₁ corresponds to 20 March 2024, and the harvest at t₂ corresponds to the accumulated biomass from the seven cuts.

Reproductive growth

Reproductive growth was evaluated on the 26 plants per infection level (Fig. 2). The beginning of plant flowering was defined when immature (green) spikes were visible. The caryopses were harvested as they matured and were stored in paper bags at room temperature, labeled with the plant identification and harvest date. The reproductive period for each plant was defined as the time (days) between the flowering beginning and the last harvest of mature caryopses (Fig. 2). On 5 March 2025 (three months after the last harvest), the mature spikes were threshed, and the caryopses were weighed. The allocation of plant resources to reproduction was determined through reproductive effort; that was calculated as the weight of caryopses per plant divided by the total above-ground biomass (leaf blade, leaf sheaths, and panicle) per plant. Thousand-caryopsis weight was determined by counting and weighing caryopses samples in ten genotypes. For each genotype, three replicates of

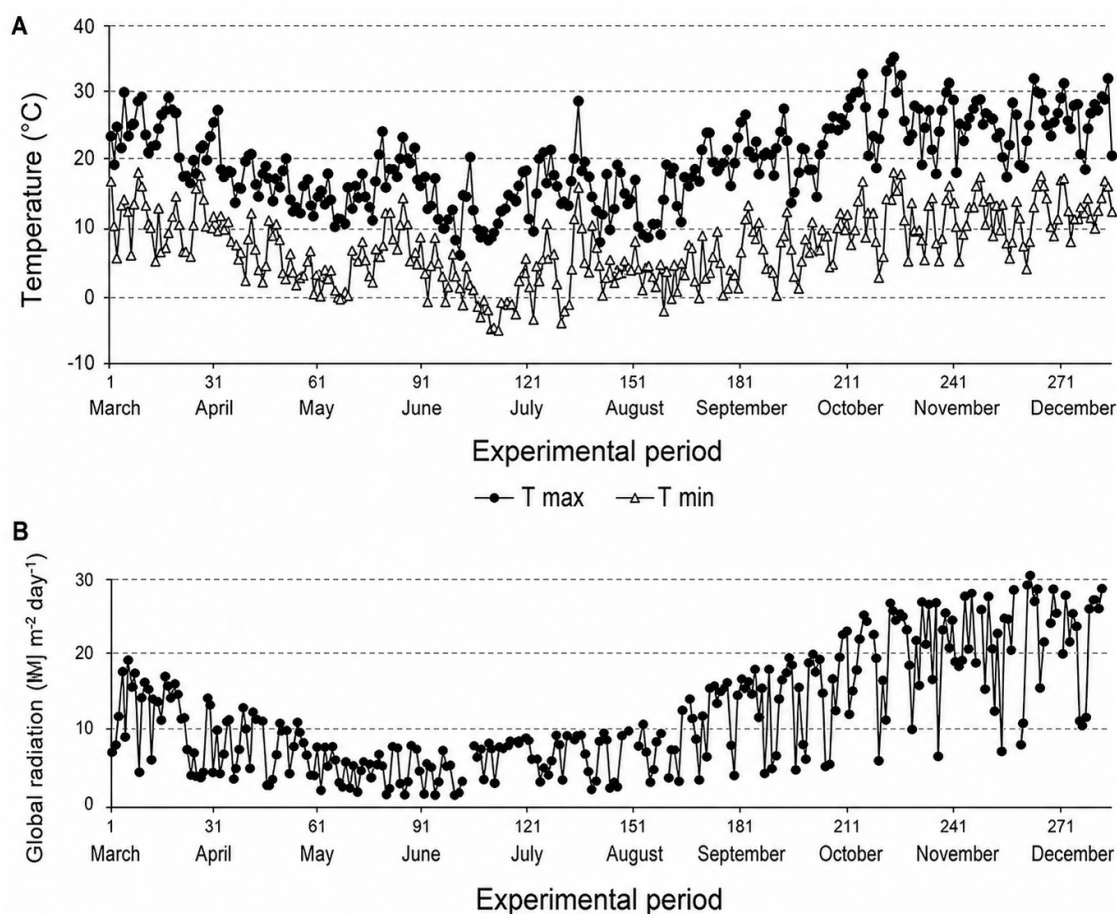


Fig. 3. A: Maximum and minimum daily air temperature, T max and T min, respectively. **B:** Global daily radiation recorded during the experimental period, year 2024.

100 randomly selected caryopses were prepared and weighed using an analytical balance with a precision of 0.0001 g. The mean weight of the 100-caryopsis replicates was calculated and multiplied by 10. In total, 60 replicates were analyzed (10 genotypes x 2 endophyte levels x 3 replicates).

On 7 March 2025, germination energy and germination power were evaluated following ISTA (2020). Ten plants at each infection level were randomly selected (samples). Fifty caryopses per sample were placed in rolls of sterilized absorbent paper. The paper was soaked with approximately 20 ml of distilled water applied using a sprayer. The 20 rolls (2 infection levels x 10 samples) were left in a vertical position in individual glasses, which were placed in a germination chamber for 14 days at 20 °C with a photoperiod of 8 h and photon flux density of 18 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Caryopses were considered germinated when the radicle reached ≥ 2 mm in length. Germination energy was recorded after 7 days and was calculated as the percentage of germinated caryopses relative to the total number of sown caryopses (50). According to ISTA (2020) classification, germinated and dead caryopses were determined, as well as normal and abnormal seedlings were recorded. Germination power was recorded at 14 days as normal seedlings/total caryopses; then, the length of primary root and coleoptile was measured using a millimetric ruler on a random sample of 10 normal seedlings per infection level. Normal seedlings indicate their ability to become plants under favorable conditions. Normal seedlings were those that become normal plants (with root, hypocotyl, coleoptile, and primary leaves) under favorable

conditions. Abnormal seedlings were those that exhibited any deterioration of the tissue and were not classified as normal.

Endophyte transmission

Endophyte transmission from the maternal plant to the caryopses was determined in the seedlings. Seedlings from E+ caryopses were grown in pots. Thirty days later, the presence of the fungus was determined by microscopic observation of hyphae between parenchyma cells in the leaf sheaths of the seedlings (Belanger, 1996).

Statistical analysis

The data were analysed statistically using the Student's t-test ($\alpha = 0.05$). The results are presented on a table and in figures. The fitness variables are presented in a figure showing the average values of the number of caryopses and the total above-ground vegetative biomass per plant (leaf, leaf sheaths, and panicle). The figure consists of four quadrants and allows to identify the genotypes with values above or below of the average for each variable. This approach is appropriate to identify plant performance under different growing conditions (Buraschi *et al.*, 2024). All analyses were performed using Statistica 6.0 (StatSoft Inc., USA).

RESULTS

Vegetative growth

At the beginning of the experiment, tiller number per plant did not differ significantly ($P = 0.06$) with the level of infection, being in average ($\pm$ S.E.) 14.80 ± 1.73 tillers pl^{-1} . Above-ground biomass did not differ ($P = 0.07$) between infection levels, with an average value of 4.25 ± 0.52 g plant^{-1} . Root biomass was significantly higher ($P = 0.03$) in E- than in E+ plants, 4.73 ± 0.71 g plant^{-1} and 2.36 ± 0.27 g plant^{-1} , respectively. No plant mortality was recorded during the experimental period.

At the end of the cutting, above-ground vegetative biomass accumulated through successive harvests (seven) was slightly higher ($P = 0.06$) in E+ than in E- plants. The relative growth rate (RGR) was significantly higher in E+ than in E- plants (Table 1).

Reproductive growth

All plants reached the reproductive stage. Plant flowering of both infection levels occurred between October and November 2024 (Fig. 3). No differences in degree days were recorded for the flowering beginning ($P = 0.63$) and the last caryopses harvest ($P = 0.19$) between infected and uninfected plants (Fig. 4).

Table 1. Average values ($\pm$ SE) of the vegetative and reproductive variables recorded in tall fescue plants. The abbreviations are: pl, plant; RGR, relative growth rate. The same letters for a same variable indicate no significative differences ($p < 0.05$) between plants uninfected (E-) and infected (E+) by *Epichloë coenophiala* endophyte. References= *: shoot biomass accumulated through successive harvests.

Traits	Plant infection level	
	E +	E -
Shoot vegetative biomass (g pl^{-1})*	5.38 ± 0.27 a	4.66 ± 0.26 a
RGR (1 week $^{-1}$)	0.058 ± 0.003 a	0.043 ± 0.002 b
Reproductive period (days)	54.08 ± 1.78 a	56.88 ± 1.38 a
Reproductive effort (%)	22.22 ± 150 a	21.10 ± 1.81 a
1000 caryopses (g)	2.19 ± 0.03 a	2.24 ± 0.07 a
Panicle (N pl^{-1})	15.65 ± 1.37 a	15.61 ± 1.65 a
Caryopses (g pl^{-1})	2.63 ± 0.21 a	2.28 ± 0.23 a
Germination energy (%)	89.40 ± 2.97 a	91.60 ± 2.09 a
Germination power (%)	84.80 ± 2.67 a	89.20 ± 3.82 a
Primary root (cm)	5.82 ± 0.44 b	5.32 ± 0.40 a
Coleptile (cm)	9.96 ± 0.57 a	10.17 ± 0.52 a

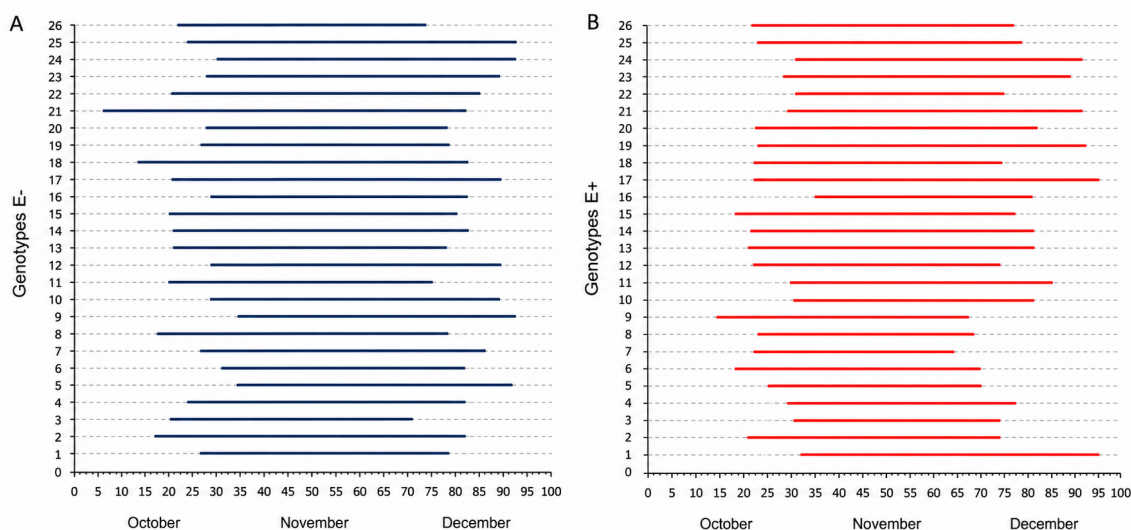


Fig. 4. Period between flowering beginning and the panicle harvest with mature caryopses in tall fescue plants. **A:** Uninfected by *Epichloë coenophiala* endophyte (E-). **B:** Infected by *E. coenophiala* endophyte (E+). Plants are arranged from 1 to 26 according to their identification.

The reproductive period did not differ significantly between E- and E+ plants, approximately 55 days (Fig. 4). However, this period varied among plants with the same level of infection. Twenty-five percent of E+ plants completed the reproductive stage in less time than E- plants. Total number of panicles per plant did not differ between E- and E+ plants (Table 1). However, the panicles matured earlier in infected than in uninfected plants (Fig. 5).

Reproductive effort and caryopses production per plant were not significantly affected by the infection level (Table 1). Sixty-two percent of E+ plants and 54 % of E- plants exhibited reproductive effort values higher than the overall average (Fig. 6). The weight of 1,000 caryopses was not significantly different between E+ and E- plants (Table 1). Caryopses number per plant was not significantly different between infection levels (Table 1), however, E+ plants produced 182 caryopses per plant more than E- ones.

Plant performance

The infected plants showed better performance. The plants with a higher number of caryopses and vegetative biomass per plant were more numerous in infected than in uninfected, 12 and 8, respectively (Fig. 7). Plants with lower performance, low vegetative and reproductive biomass, were more numerous in uninfected than in infected ones, 12 and 6, respectively (Fig. 7).

Germination

Germination energy, germination power, and coleoptile length did not vary significantly between infection levels of caryopses (Table 1). Abnormal seedling was 9.60 % and 4.60 % in infected and uninfected caryopses, respectively. Non-germinated caryopses were approximately 6.30 % in both infection levels. Length of primary seminal root was significantly greater in E+ than in E- seedlings (Table 1).

Endophyte transmission

The fungus transmission from the mother plant to the caryopses was not interrupted under defoliation conditions.

DISCUSSION

Under the experimental conditions, the presence of the endophyte did not represent a cost to the E+ plants in terms of vegetative and reproductive growth. On the contrary, the RGR of above-ground biomass and root length of the infected seedlings were higher than those of uninfected plants. Furthermore, a higher percentage of infected plants showed greater reproductive effort, total vegetative biomass and caryopsis number than uninfected

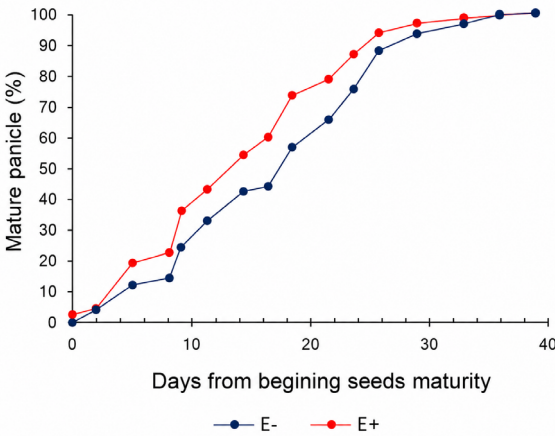


Fig. 5. Accumulative panicle of tall fescue plant harvest with mature caryopses. References= E+: plant infected by *Epichloë coenophiala* endophyte; E-:uninfected plants.

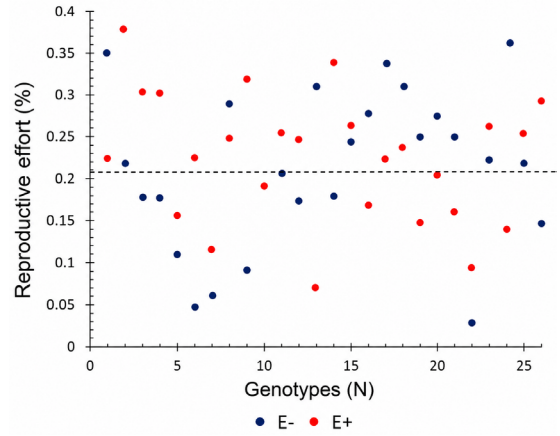


Fig. 6. Reproductive effort of tall fescue plants. The dashed horizontal line indicates the average reproductive effort of all plants. References= E+: plant infected by *Epichloë coenophiala* endophyte; E-: uninfected plants.

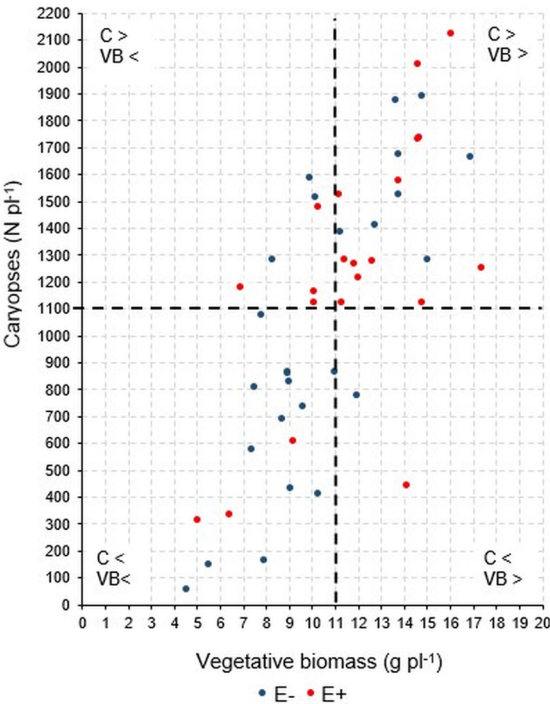


Fig. 7. Tall fescue plant performance. References: The dashed horizontal and vertical lines indicate the average of caryopses number and total vegetative biomass of all plants. References= E+: plant infected by *Epichloë coenophiala* endophyte; E-: uninfected plants; C: caryopses number per plant; VB: vegetative biomass per plant.

ones. These results, together with the observation that the fungal transmission was not affected by defoliation, suggest that the endophyte confers greater fitness to the host plants and resilience (Mooney & Hobbs, 1986; Vila-Aiub *et al.*, 2005; Gundel *et al.*, 2008; Iannone *et al.*, 2012; Saikkonen *et al.*, 2016).

Vegetative and reproductive growth

The defoliation frequency applied in this experiment was higher than that used in previous studies. Previously, we reported that under a defoliation frequency of 450 °C days, infected tall fescue plants produced fewer panicles than those cut every 850 °C days (Petigrosso *et al.*, 2021b). Tall fescue plants growing under defoliation every two weeks during the vegetative stage showed reduced panicle production in E+ plants compared to plants growth under control conditions (Petigrosso *et al.*, 2021b). García Parisi *et al.* (2012) reported that under defoliation, infected plants of *L. multiflorum* experienced a cost, exhibiting lower vegetative and reproductive growth. Consistent with these results, Ueno *et al.* (2024) reported under defoliation, a metabolic cost of endophyte presence in tall fescue plants, evaluated in terms of vegetative growth during post-defoliation recovery. Although no significant

differences in caryopses production were detected, infected plants produced more caryopses (182 caryopses/plant) and greater above-ground biomass than E- plants (Fig. 7). Because the endophyte did not impose a cost on vegetative growth, the higher RGR in E+ plants was due to both genotype and the presence of fungus.

Although the reproductive period was comparable between infected and uninfected genotypes (55 days), it varied among plants within the same infection level (Fig. 4). Twenty-five percent of E+ plants completed their reproductive period in a shorter time than E- plants. A short life cycle and asynchronous reproduction are attributes commonly observed in invasive plant species (Li *et al.*, 2018).

Germination and transmission of endophyte

Germination energy, germination power, and coleoptile length did not vary with endophyte presence. These traits may be affected more by incubation conditions than by the presence of the endophyte (Gundel *et al.*, 2006). However, in previous studies infected caryopses have shown greater germination energy under abiotic stresses conditions, as salinity with NaCl, than uninfected caryopses (Thomas *et al.*, 2022). Petigrosso *et al.* (2021b) found that the seedling emergence of tall fescues from infected caryopses was significantly higher than that from uninfected ones under favorable conditions. This result is consistent with that reported by Novas *et al.* (2003), who found that the number of seedlings emerged of *Bromus setifolius* was higher from infected caryopses with *Neotyphodium* sp. than from uninfected ones. In our experiment, infected seedlings produced longer primary roots than uninfected ones. Root development in seedlings is an attribute that influences plant establishment and subsequent growth (Baskin & Baskin, 2014; Petigrosso *et al.*, 2021a,b). For example, Dziamski *et al.* (2012) working with six grass species, including *Festuca arundinacea*, reported that seedling root development was positively correlated with adult plant biomass. Under conditions of low fertility or water stress, the allocation of resources to produce a high specific root length (length/biomass) can improve the performance of adult plants (Grossnickle & Ivetić, 2022). Consistent with these results, Clay (1987) reported that

seedlings of *Lolium perenne* and *L. arundinaceum* from caryopses infected with *Epichloë* sp. performed better than uninfected seedlings in terms of germination rates and vegetative growth. Shearin *et al.* (2018) suggested that the spread of an exotic grass, *Phragmites australis*, in different plant communities in the United States of America may be related to the presence of the fungus in the caryopses; the endophyte promoted germination and seedling growth. Another example was reported by Ahlholm *et al.* (2002), who found that the endophyte promoted seedling growth of *Festuca* spp. during the early stage of establishment. Comparable results were reported by Uchitel *et al.* (2011) who sowed, in different plant communities, *Lolium multiflorum* caryopses uninfected and infected with the endophyte *Epichloë occultans* (formerly referred to as *Neotyphodium occultans*). Seedling recruitment and plant reproduction were higher from infected caryopses than from uninfected ones. In addition, the authors noted that infected caryopses were less preferred (bitten) by resident granivorous. In tall fescue has been documented that small granivorous such as birds (Madej & Clay, 1991) and ants, like *Pogonomyrmex* spp. (Knoch *et al.*, 1993), showed less preference for infected caryopses than for uninfected ones. Future studies should investigate whether the granivorous of the grasslands prefer or reject tall fescue caryopses depending on their level of infection. This subject could also help to explain the spread of tall fescue populations in different environmental conditions, such as pastures, rural roadsides, and grasslands.

Defoliation did not cause failures in the transmission of the endophyte from the mother plant to the caryopses; in other words, all infected plants produced infected caryopses, which is consistent with the results of previous studies (Petigrosso *et al.*, 2020). Under less intense defoliation regimes and flooding conditions, vertical transmission of endophyte to caryopses was not affected (Petigrosso *et al.*, 2026a). These results indicate that, under defoliation conditions, the endophyte can be transmitted from generation to generation. The close link between endophyte fitness and its host grass is mediated through a mutualistic cooperation that confers advantages to both partners (Clay & Schardl, 2002; Iannone *et al.*, 2012; Saikkonen *et al.*, 2016). This study provides

experimental evidence that, under defoliation, the symbiosis between tall fescue and *E. coenophiala* does not impose a cost on the host plant. The endophyte may enhance key functional traits that promote plant persistence and propagation in the Pampas grasslands.

CONCLUSIONS

Under the defoliation conditions applied in this study, the presence of *E. coenophiala* did not affect the vegetative and reproductive growth of tall fescue plants. Defoliation did not result in failure of endophyte transmission. Infected plants had a higher relative growth rate in above-ground biomass, and their seedlings had longer roots than uninfected plants. In addition, E+ plants were more abundant in total shoot vegetative biomass and caryopses number than E- plants. These traits and those analyzed in previous experiments, confer enhanced fitness to infected plants, consistent with their capacity of propagation in the Pampas region.

AUTHOR CONTRIBUTIONS

All authors equally contributed to data collection, data interpretation, and manuscript writing.

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