CSIRO PUBLISHING

Crop & Pasture Science, 2011, **62**, 1010–1016
http://dx.doi.org/10.1071/CP11300

Interaction between plant genotype and the symbiosis with *Epichloë* fungal endophytes in seeds of red fescue (*Festuca rubra*)

P. E. Gundel[A,B,D], *I. Zabalgogeazcoa*[C], *and B. R. Vázquez de Aldana*[C]

[A]IFEVA-Facultad de Agronomía (UBA)/CONICET, Cátedra de Ecología, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina.
[B]MTT Agrifood Research Finland, Plant Protection, 31600 Jokioinen, Finland.
[C]Instituto de Recursos Naturales y Agrobiología de Salamanca, IRNASA-CSIC, Apartado 257, 37071 Salamanca, Spain.
[D]Corresponding author. Email: gundel@agro.uba.ar

Abstract. In diverse natural habitats of Europe, plants of *Festuca rubra* are commonly infected by the fungal endophyte *Epichloë festucae*. Under several circumstances, the association between the grass and the fungus has been shown to be mutualistic. Here, we conducted an experiment to study the differences in seed germination and mortality between infected (E+) and endophyte-free plants (E–) at different temperatures (12 and 25°C) and water potentials (0 and –0.5 MPa). Three half-sib lines of *F. rubra*, each composed of E+ and E– seeds, and derived from infected plants from semiarid grasslands were used. Although the endophyte effect depended on the incubation condition, germination percentage was significantly greater for E– (52%) than for E+ seeds (41%). Seed germination was more inhibited by the low water potential (75 *v.* 24% for –0.5 and 0.0 MPa, respectively), than by the high temperature (64 *v.* 35% for 25 and 12°C, respectively). However, mortality was highly dependent on the interaction between plant genotype and endophyte, and between temperature and water condition. It is remarkable that while highly dependent on the host genotype, there was a clear effect of endophyte increasing seed survival, especially in those treatments that were unfavourable for germination. For example, in the more restrictive treatment (25°C and –0.5 MPa), seed survival was on average, 44 and 39% for E+ and E–, respectively. In general, the endophyte affected seed characteristics of *F. rubra* by reducing the percentage of germination, but simultaneously increasing seed survival.

Additional keywords: dehesas, grasslands, plant–endophyte symbiosis, seed germination, seed survival, symbiotic interaction.

Received 4 May 2011, accepted 11 November 2011, published online 13 December 2011

Introduction

Plants and animals have evolved in interaction with myriad of micro-organisms such as bacteria, fungi, and viruses. As a result of certain symbiotic interactions, macro-organisms have been able to extend their ecological niche (e.g. plant–mycorrhizal interaction), while in other cases, they have suffered a permanent threat (e.g. plant–pathogen interaction). Symbioses between cool-season grasses and fungal endophytes of the *Epichloë* or *Neotyphodium* genera constitute an interesting case of study because depending on the partners' species, genotype, and ecological context, it may range from pathogenic to mutualistic (Clay and Schardl 2002; Saikkonen *et al*. 2004). The replacement of host reproductive structures by a fungal stroma during sexual reproduction, a phenomenon known as choke disease, is the pathogenic symptom of some *Epichloë* species (e.g. *E. typhina*; Clay and Schardl 2002). However, sexual reproduction of the fungus never occurs in *Neotyphodium* and rarely in several *Epichloë* species. In these cases, the fungus relies on vertical transmission for reproduction and dispersal. Given that plants infected by asymptomatic endophytes have been found to show increased resistance to herbivores and other biotic and abiotic stress factors (Malinowski and Belesky 2000; Clay and Schardl 2002), endophytes are being considered in many breeding programs of forage and turf grasses (Easton 2007). Therefore, it is important to understand the underlying mechanisms of these context-dependent outcomes for the applied use of the grass–endophyte symbiosis.

The positive effects of endophyte infection on host plant performance are usually explained by mechanisms such as alkaloid-mediated resistance to herbivory, enhanced nutrient uptake, or higher tolerance to oxidative stress (Malinowski and Belesky 2000; Clay and Schardl 2002; White and Torres 2010).

Nonetheless, some effects of endophytic fungi on host growth and persistence have been elusive. In fact, several works have recorded neutral or negative effects of the symbiosis on different plant traits or growth stages. For example, under a low resource level, endophyte presence has been found to depress plant performance in populations of tall fescue (*Lolium arundinaceum*), meadow fescue (*Festuca pratensis*) and perennial ryegrass (*Lolium perenne*) (Ahlholm *et al*. 2002; Cheplick 2007; Marks and Clay 2007), but had no effect on vegetative biomass production in red fescue (*Festuca rubra*), perennial ryegrass or *Brachypodium phoenicoides* (Spiering *et al*. 2006; Zabalgogeazcoa *et al*. 2006*a*, 2008). When the performance of endophyte-infected plants is lower than that of non-infected ones in the same environment, the endophyte demand for carbohydrates and nutrients from the host can be considered as a cost, and the symbiosis turns into parasitism (*s.l.* Ahlholm *et al*. 2002; Spiering *et al*. 2006; Cheplick 2007). Furthermore, the negative effect of endophyte on seed germination and longevity are other negative effects not directly caused by the fungus as a nutrient sink (Gundel *et al*. 2006, 2009, 2010; Spiering *et al*. 2006). Therefore, the endophyte effect on host plant performance is not only dependent on the ecological context determined by the partners' genotype and environmental conditions, but also on the plant developmental stage (Ahlholm *et al*. 2002; Saikkonen *et al*. 2004; Spiering *et al*. 2006; Wäli *et al*. 2009).

Less studied than other fitness traits, seed and seedling traits (e.g. seed size, weight, or germination, and seedling recruitment) have been pointed out as critical in the interaction between host plants and endophyte symbionts (Neil *et al*. 2003; Gundel *et al*. 2006, 2010; Wäli *et al*. 2009). Seed germination requirements are important for the ecology and agricultural performance of species since they control phenology and uniformity of plant stands, essential for successful extensive cropping, forage farming, horticulture, and turfs (Harper 1977; Martínez-Ghersa *et al*. 2000; Bradford 2002). Synchronised germination may be related to uniform seedling establishment and competitive performance, while seed dormancy is a risk-avoidance trait that may be important for surviving future unfavourable conditions (Benech-Arnold *et al*. 2000; Martínez-Ghersa *et al*. 2000; Bradford 2002). Especially in semiarid environments, temperature and water availability appear as the main factors controlling seed germination and dormancy (Allen *et al*. 2000; Martínez-Ghersa *et al*. 2000; Bradford 2002). Seed dormancy level for a given population can be easily visualised as how narrow or wide is the range of environmental conditions that allow seeds to germinate (Benech-Arnold *et al*. 2000). Seed responses to water availability and temperature may be dependent on species, population, and factors such as environmental conditions during seed growth and development, after-ripening or storage conditions, and the symbiotic association with endophytic fungi (Martínez-Ghersa *et al*. 2000; Bradford 2002; Neil *et al*. 2003; Faeth *et al*. 2004; Gundel *et al*. 2006).

Festuca rubra L. belongs to a complex of fine fescues that is native to many parts of Europe (Arroyo García *et al*. 2002; Bazely *et al*. 2007; Wäli *et al*. 2009). It is a very important grass used as forage in natural and managed grasslands and also as turf. For example, in savannah-like semiarid grasslands of western Spain known as 'dehesas', it is an important forage resource (Vázquez de Aldana *et al*. 2008). Natural populations of this species are typically infected by the endophytic fungus *Epichloë festucae* (Leuchtmann *et al*. 1994). Although the incidence of infected plants can vary due to grazing level and altitude (Bazely *et al*. 2007) incidences close to 70% have been observed in habitats as different as semiarid grasslands and marine cliffs (Zabalgogeazcoa *et al*. 1999, 2006*b*; Arroyo García *et al*. 2002). Positive effects of this endophyte like increased herbivore and disease resistance, and nutrient content have been reported (Ahlholm *et al*. 2002; Zabalgogeazcoa *et al*. 2006*a*; Clarke *et al*. 2006); however, some effects on host seeds and seedlings have been variable (Wäli *et al*. 2009). *F. rubra* is an important turfgrass species, and *E. festucae* is being used for the improvement of turfgrass cultivars (Brilman 2005); at the present time numerous endophyte-infected turfgrass cultivars are commercially available worldwide.

Considering that the seed stage is critical for plant survival, and that high endophyte infection rates have been observed in natural populations we expected that endophyte infection could have an effect upon seed germination and survival. Therefore, the objective of this study was to evaluate the effects of the infection by *E. festucae* on seed weight, germination and mortality in three different genotypes of *F. rubra* under different temperature and water availability conditions.

Materials and methods

Plant material

Three lines of half-sibs of *F. rubra* (RAB, SAN, and PEN), each consisting of endophyte-infected (E+) and endophyte-free (E–) plants were used for the experiment. Each line was developed from a single *F. rubra* plant originally infected by *E. festucae* (Zabalgogeazcoa *et al*. 2006*a*). The mother plants were sampled at three locations, 40 km apart in semiarid grasslands (dehesas) of the province of Salamanca, in western Spain. Each mother plant was divided in six ramets, and half of them were treated with a systemic fungicide to kill the endophyte. After verifying the infection status, fungicide treated (E–) and non-treated (E+) ramets were transplanted to the field in a research farm in Salamanca. Seeds produced by these plants were harvested, cleaned and stored at 10°C until they were used. Endophyte infection frequency in each population was determined by inspecting stained seeds ($n = 30$) by light microscopy (Bacon and White 1994). Results of infection were 100% for E+ biotypes, except for RAB, which was 96,6%, and 0% for all the E– biotypes.

Measurements and experiments

Seed weight

Twelve samples (30 seeds each) of RAB, SAN and PEN and endophyte infection status (E+ and E–) were weighed in order to estimate the 1000-seed weight.

Germination experiment

Seed germination as affected by plant genotype and the endophyte was evaluated under different conditions of temperature and water availability since these two factors are the most important controlling seed germination in nature (Benech-Arnold *et al.* 2000; Martínez-Ghersa *et al.* 2000; Bradford 2002). The germination experiment consisted of a four-way factorial arrangement within a randomised design with six replications. The factors were: genotype (RAB, SAN and PEN), endophyte (E+ and E–), water availability (0 and –0.5 MPa), and temperature (12 and 25°C). The two water potentials were obtained with distilled water (0 MPa) and a polyethylene-glycol (PEG-6000) solution (–0.5 MPa; Michel 1983). For the temperature treatments, two different growth chambers set at 12 or 25°C, and periodically checked with a digital thermometer, were used. The non-independence between replicates within chambers was alleviated by placing the dishes at random and by changing the positions every day throughout the experiment (Morrison and Morris 2000). The experimental unit was a 9-cm Petri dish containing 30 seeds that were sown on a germination paper disk (Filter Paper, Whatman GmbH, Germany). Each dish was soaked with 5 mL of water or PEG solution, depending on the treatment. Before sowing, the seeds were surface-disinfected by immersion for 10 min in a 50% (v/v) commercial bleach solution (2.5% active chlorine). Seed germination was recorded as radicle protrusion every 2 days, and germinated seeds were removed from the Petri dish. The seed counting was done for a period of 2 weeks, until the germination dynamics reached a plateau; after that, all seeds were transferred to fresh distilled water and incubated at 12°C, even those that already were in this condition. In the new condition, seeds were allowed to germinate for 2 additional weeks, and this procedure enabled to distinguish dead from live seeds that were inhibited by the high temperature or PEG solution. Ungerminated seeds with soft consistency and disease symptoms (dark fungal mycelium growing on them) were considered dead. Thus, three seed categories were considered: germinated seeds, dormant or inhibited seeds, and dead seeds. Seed germination was estimated as the ratio of germinated seeds to live seeds (germinated seeds + inhibited seeds), while the proportion of dead seeds (mortality) was estimated as the ratio of dead to the total seeds sown in the dish.

Data analysis

Differences in 1000-seed weight as affected by the endophyte infection status were analysed by means of a Student's *t*-test for each plant genotype. Effects of host plant genotype, endophyte infection status, incubation temperature, and water potential on total seed germination and seed mortality were analysed with a four-way ANOVA. Normality and homogeneity of variances were accomplished by $\log_{10}$ transformation of data only when necessary. For significant interactions, the difference between means of E+ and E– plants of each line were tested with an l.s.d. procedure at $P < 0.05$. All data analyses were performed with R software (R Development Core Team 2010).

Results

The endophyte infection status affected seed weight depending on the plant genotype. Non-infected seeds were significantly heavier than infected seeds in the RAB genotype, but there were no differences associated to the infection in the SAN or PEN genotypes (Table 1).

Regarding seed germination, the ANOVA showed three significant three-way interactions: plant genotype × endophyte × temperature; plant genotype × temperature × water potential; and endophyte × temperature × water potential (Table 2). Beyond the particularities associated to each plant genotype, seed germination was affected by endophyte infection, incubation temperature, and water potential (Table 2), being inhibited at the higher temperature (25°C), the lower water potential (–0.5 MPa), and in endophyte-infected seeds for RAB and PEN genotypes (Fig. 1).

At the temperature of 12°C, seed germination of PEN genotype was significantly greater for E– than for E+ under both water potentials. The same result was observed in RAB genotype, but only under the low water potential, while no effect of the endophyte was found in the SAN genotype under both water potentials (Fig. 2). The overall proportion of germination and the mean difference between E+ and E– seeds tended to decrease at the higher temperature (Fig. 2). At 25°C, the difference in germination between E+ and E– seeds was significant only for line SAN. At this temperature and the lower water potential (–0.5 MPa) germination was greatly

Table 1. 1000-seed weight (g) of three genotypes of *Festuca rubra* (RAB, SAN, PEN) infected (E+) and non-infected (E–) with *Epichloë festucae*

Values are averages of 12 samples of 30 seeds each, and standard errors are shown in parentheses. Means of E+ and E– seeds of each line were compared with a Student's *t*-test

Plant genotype	Endophyte infection status E+	E–	*P*-value
RAB	0.99 (0.01)	1.06 (0.02)	0.009
SAN	0.94 (0.01)	0.93 (0.01)	0.849
PEN	1.37 (0.01)	1.38 (0.02)	0.775

Table 2. Summary of ANOVA of the effects of plant genotype (RAB, SAN, PEN), endophyte infection status (E+ and E–), incubation temperature (12 and 25°C) and water potential (0 and –0.5 MPa) on *Festuca rubra* seed germination and seed mortality

For the analysis of seed germination dead seeds were excluded

Effect	d.f.	Seed germination *F*	*P*-level	Seed mortality *F*	*P*-level
Plant genotype (L)	2	2.53	0.083	7.79	<0.001
Endophyte (E)	1	32.60	<0.001	12.23	<0.001
Temperature (T)	1	202.00	<0.001	112.95	<0.001
Water potential (W)	1	645.00	<0.001	0.30	0.584
L × E	2	9.30	<0.001	4.72	0.010
L × T	2	6.59	0.002	3.37	0.037
E × T	1	0.36	0.548	2.27	0.134
L × W	2	0.90	0.408	0.23	0.791
E × W	1	0.02	0.895	3.43	0.066
T × W	1	15.80	<0.001	4.51	0.035
L × E × T	2	10.50	<0.001	0.81	0.446
L × E × W	2	0.69	0.503	0.15	0.859
L × T × W	2	4.07	0.019	0.51	0.602
E × T × W	1	8.71	0.004	3.18	0.077
L × E × T × W	2	1.11	0.333	1.53	0.220

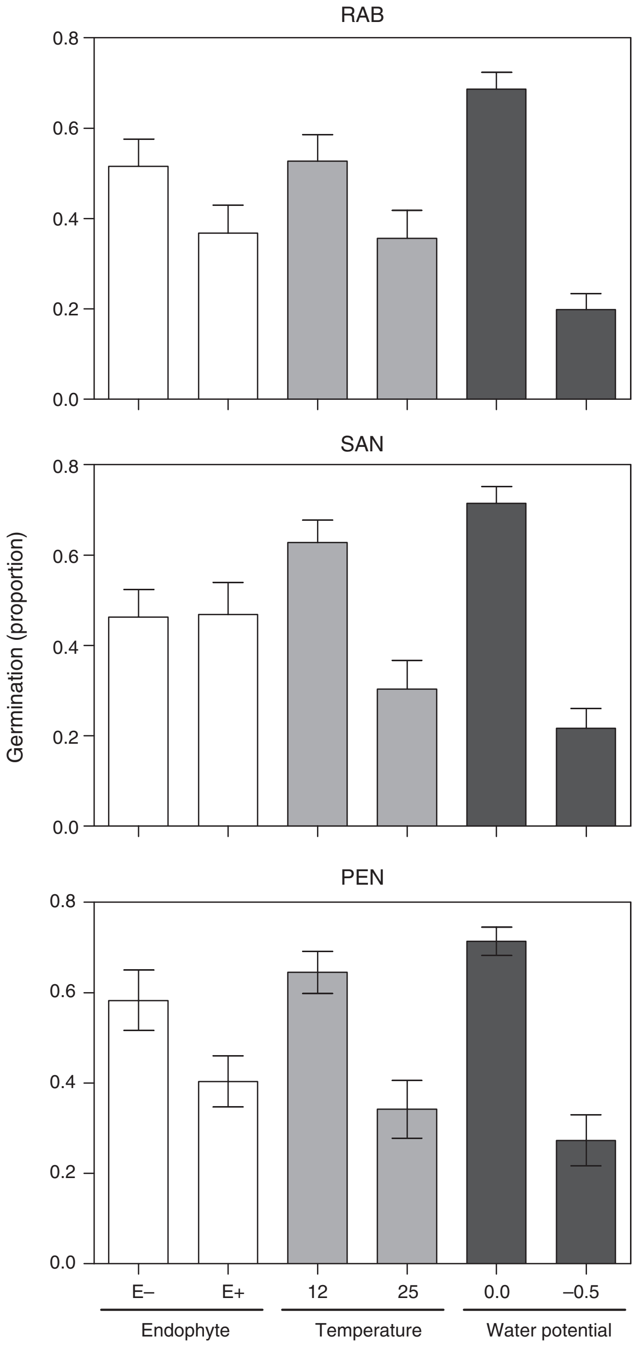

Fig. 1. Total seed germination for the three genotypes of *Festuca rubra* (RAB, SAN and PEN) as affected by endophyte infection status (E+ and E–; white bars), incubation temperature (12 and 25°C; grey bars) and water potential (0.0 and –0.5 MPa; dark bars). Vertical bars are standard errors of means.

inhibited, and differences between E+ and E– seeds were not significant.

Despite the interaction with plant genotype, in general the presence of *Epichloë* diminished the proportion of dead seeds during the experiment (Fig. 3). The ANOVA detected three significant two-way interactions on seed mortality: temperature × water potential, plant genotype × temperature, and plant genotype × endophyte (Table 2). The positive effect of endophyte improving seed survival was most evident in the treatment that had the highest inhibitory effect on seed germination: 25°C and –0.5 MPa (Fig. 3). Nevertheless, at 12°C and both water potentials the effect of the endophyte on seed mortality was significant only for the RAB genotype, and this pattern vanished under the combination of 25°C and 0.0 MPa (Fig. 3).

Discussion

A successful establishment depends on multiple factors, like the maintenance of seed quality during the quiescent state, sensing the right moment and place to germinate (dormancy), pest and disease resistance, and the rate of germination and seedling growth (Harper 1977; Benech-Arnold *et al*. 2000; Martínez-Ghersa *et al*. 2000; Bradford 2002). We found a tendency of endophyte-infected seeds to be more inhibited than non-infected ones. However, the higher level of dormancy in endophyte-infected seeds was clearly dependent on the host genotypes, varying in a complex way with the incubation conditions. This is in accordance with results by Wäli *et al*. (2009), who observed differences in the germination of *F. rubra* seeds dependent on the habitat where mother plants were collected. The inhibitory effect of endophyte on seed germination is consistent with previous studies on *Lolium multiflorum*, where the hydrotime model indicated a higher (less negative) base water potential for *Neotyphodium occultans*-infected than non-infected seeds (Gundel *et al*. 2006). In contrast with these results, endophyte infection did not affect the timing or the percentage of germination in *F. arizonica* seeds (Neil *et al*. 2003; Faeth *et al*. 2004). The increased survival rate observed in E+ imbibed seeds is also consistent with the observations reported for *L. multiflorum* (Gundel *et al*. 2006). During our experiment, moulds developed on some ungerminated seeds, and it is possible that *E. festucae* may enhance seed resistance against pathogens. This endophyte is known to improve resistance against some fungal diseases in adult *F. rubra* plants (Clay and Schardl 2002; Clarke *et al*. 2006).

In the dehesa natural grasslands where the plants used in this work were obtained, ~70% of the plants of *F. rubra* are associated with *E. festucae* (Zabalgogeazcoa *et al*. 1999; Arroyo García *et al*. 2002). These habitats have a remarkable floristic complexity, and a Mediterranean climate with precipitations occurring mainly in spring and autumn, and severe droughts in summer (Vázquez de Aldana *et al*. 2008). Therefore, although plant competition is very likely to affect the fitness of *F. rubra*, seed persistence in the soil seed bank can be a very important fitness trait for the species. Although precipitations are concentrated in spring and autumn, occasional rain events can happen any time throughout the year (Vázquez de Aldana *et al*. 2008), providing enough water to trigger germination. Therefore, considering that seed ripening and shedding occur in late spring, and that fungal alkaloids in seeds have been found to be an effective deterrent against granivorous animals (Clay and Schardl 2002), seed persistence during summer might be a very important fitness trait, perhaps more than readily germination. If the greater

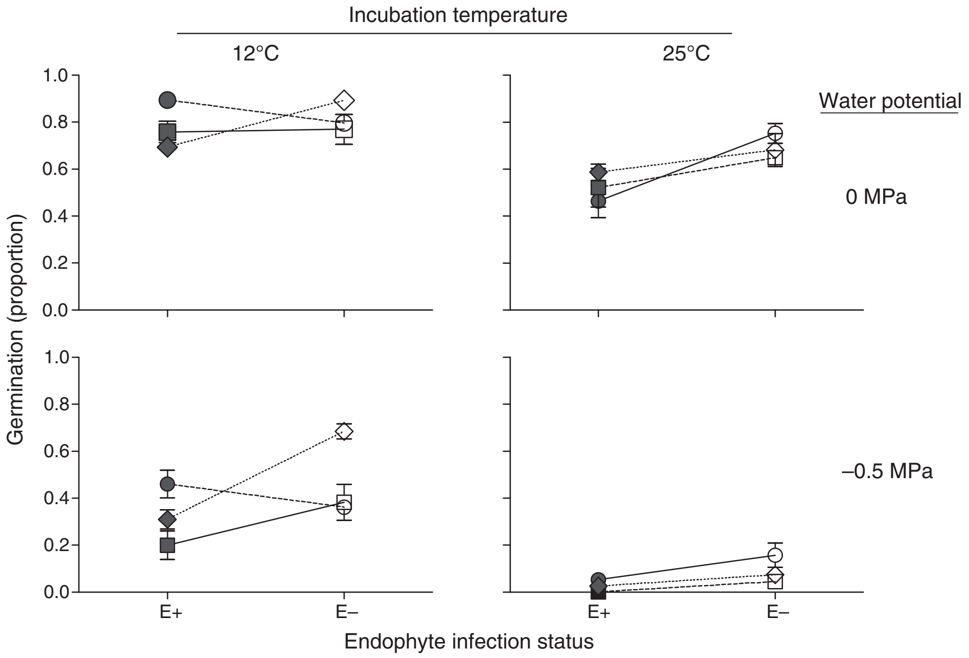

Fig. 2. Norm of reaction graphs showing the total seed germination reached by the three genotypes of *Festuca rubra* [RAB (□), SAN (○) and PEN (◇)] as affected by endophyte infection status [E+ (dark symbols) and E– (white symbols)] at two constant temperatures (12 and 25°C) and two water potentials (0 and –0.5 MPa). Values are averages of six samples of 30 seeds each.

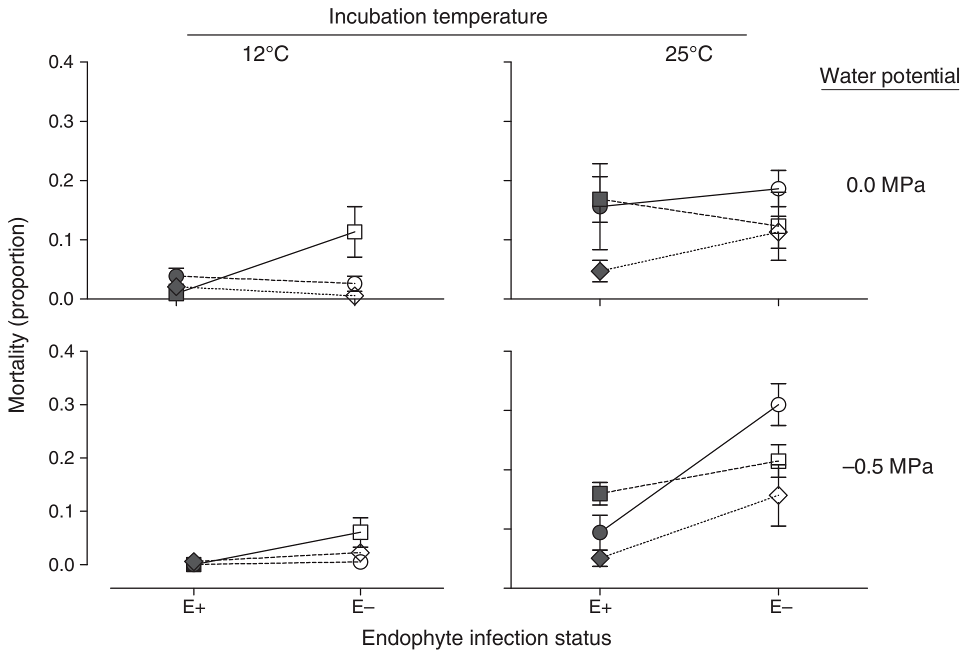

Fig. 3. Norm of reaction graphs showing the seed mortality observed in the three genotypes of *Festuca rubra* [RAB (□), SAN (○) and PEN (◇)] as affected by endophyte infection status [E+ (dark symbols) and E– (white symbols)] at two constant temperatures (12 and 25°C) and two water potentials (0 and –0.5 MPa). Values are averages of six samples of 30 seeds each.

survival of endophyte-infected seeds were a fitness trait for *F. rubra* in semiarid dehesa grasslands, our results would contribute to explain the higher frequency of endophyte-infected plants with respect to non-infected ones in this ecosystem (Zabalgogeazcoa *et al*. 1999; Arroyo García *et al*. 2002).

The fact that in this experiment seed germination was significantly greater at 12°C than at 25°C contrasts with the results of other studies of germination in *F. rubra*. Brar and Palazzo (1997) did not find differences at 15 and 25°C in the germination rates of commercial varieties of *F. rubra*, and Larsen and Bibby (2005) reported that the final germination of *F. rubra* subsp. *littoralis* was little affected by temperature in a range between 8 and 24°C. Germination inhibition by high temperatures could result from the existence of a remaining level of primary dormancy or by the re-induction of secondary dormancy (Benech-Arnold *et al*. 2000). This preference for the lower temperature in the *Festuca* genotypes from semiarid grasslands might be a result of adaptation to germinate in the early spring and autumn, when the seasonal rains occur. When temperatures increase in the late spring and summer, rainfall is unusual in this habitat, and seedling recruitment would be more unlikely. The variation in germination rates observed in *F. rubra* genotypes from different habitats (Wäli *et al*. 2009) might result from adaptations to increase seedling survival in particular environments.

Together with other positive effects of *E. festucae* on *F. rubra* host plants, like increased resistance to herbivory and diseases, and nutrient content (Wilkinson *et al*. 2000; Clarke *et al*. 2006; Zabalgogeazcoa *et al*. 2006*a*), the increased viability of seeds supports the mutualistic nature of this grass–endophyte interaction, and this result adds interest to the use of endophytes for the improvement of turfgrass cultivars. In contrast with the undesirable toxic effect of fungal alkaloids on cattle, toxicity can be a quality feature for turfgrasses. However, though the concentration of ergovaline alkaloid in endophyte-infected *F. rubra* plants from dehesa grasslands is below the toxic threshold for cattle, ~50% of the plants contain peramine, an insect deterrent alkaloid (Vázquez de Aldana *et al*. 2010). Therefore, endophytes that confer tolerance to pests and diseases, and abiotic stress factors to turf grasses, can be applied as a sustainable and environmentally friendly strategy by diminishing the use of non-renewable energy inputs such as insecticides, fungicides and fertilisers.

Acknowledgement

This research was funded by project AGL2008-01159AGR from the Spanish Ministry of Science and Innovation.

References

Ahlholm JU, Helander M, Lehtimäki S, Wäli P, Saikkonen K (2002) Vertically transmitted fungal endophytes: different responses of host–parasite systems to environmental conditions. *Oikos* **99**, 173–183. doi:10.1034/j.1600-0706.2002.990118.x

Allen PS, Meyer SE, Khan MA (2000) Hydrothermal time as a tool in comparative germination studies. In 'Seed biology: advances and applications'. (Eds M Black, KJ Bradford, J Vázquez-Ramos) pp. 401–410. (CABI Publishing: Wallingford, UK)

Arroyo García R, Martínez Zapater JM, García Criado B, Zabalgogeazcoa I (2002) The genetic structure of natural populations of the fungal endophyte *Epichloë festucae. Molecular Ecology* **11**, 355–364. doi:10.1046/j.0962-1083.2001.01456.x

Bacon CW, White JF, Jr (1994) Stains, media, and procedures for analyzing endophytes. In 'Biotechnology of endophytic fungi of grasses'. (Eds CW Bacon, JF White Jr) pp. 47–56. (CRC: Boca Raton, FL)

Bazely DR, Ball JP, Vicari M, Tanentzap AJ, Bérenger M, Rakocevic T, Koh S (2007) Broad-scale geographic patterns in the distribution of vertically-transmitted, asexual endophytes in four naturally-occurring grasses. *Ecography* **30**(3), 367–374. doi:10.1111/j.0906-7590.2007.04985.x

Benech-Arnold RL, Sánchez RA, Forcella F, Kruk BC, Ghersa CM (2000) Environmental control of dormancy in weed seed soil banks. *Field Crops Research* **67**, 105–122. doi:10.1016/S0378-4290(00)00087-3

Bradford KJ (2002) Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Research* **50**, 248–260.

Brar GS, Palazzo AJ (1997) Temperature and germination relationships of *Festuca* varieties. *Plant Varieties and Seeds* **10**, 103–111.

Brilman LA (2005) Endophytes in turfgrass cultivars. In '*Neotyphodium* in cool season grasses'. (Eds CA Roberts, CP West, DE Spiers) pp. 341–349. (Blackwell Publishing: Ames, IA)

Cheplick GP (2007) Costs of fungal endophyte infection in *Lolium perenne* genotypes from Eurasia and North Africa under extreme resource limitation. *Environmental and Experimental Botany* **60**, 202–210. doi:10.1016/j.envexpbot.2006.10.001

Clarke BB, White JF, Hurley H, Torres MS, Sun S, Huff DR (2006) Endophyte-mediated suppression of dollar spot disease in fine fescues. *Plant Disease* **90**, 994–998. doi:10.1094/PD-90-0994

Clay K, Schardl C (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* **160**(S4), S99–S127. doi:10.1086/342161

Easton HS (2007) Grasses and Neotyphodium endophytes: co-adaptation and adaptive breeding. *Euphytica* **154**, 295–306. doi:10.1007/s10681-006-9187-3

Faeth SH, Helander ML, Saikkonen KT (2004) Asexual *Neotyphodium* endophytes in a native grass reduce competitive abilities. *Ecology Letters* **7**, 304–313. doi:10.1111/j.1461-0248.2004.00578.x

Gundel PG, Maseda PH, Vila-Aiub MM, Ghersa CM, Benech-Arnold RL (2006) Effects of *Neotyphodium* fungi on *Lolium multiflorum* seed germination in relation to water availability. *Annals of Botany* **97**, 571–577. doi:10.1093/aob/mcl004

Gundel PE, Martínez-Ghersa MA, Garibaldi LA, Ghersa CM (2009) Viability of *Neotyphodium* endophytic fungus and endophyte-infected and noninfected *Lolium multiflorum* seeds. *Botany* **87**, 88–96. doi:10.1139/B08-119

Gundel PE, Martínez-Ghersa MA, Batista WB, Ghersa CM (2010) Dynamics of *Neotyphodium* endophyte infection in ageing seed pools: incidence of differential viability loss of endophyte, infected seed, and non-infected seed. *Annals of Applied Biology* **156**, 199–209. doi:10.1111/j.1744-7348.2009.00379.x

Harper JL (1977) 'Population biology of plants.' (Academic Press: London)

Larsen SU, Bibby BM (2005) Differences in thermal time requirement for germination of three turfgrass species. *Crop Science* **45**, 2030–2037. doi:10.2135/cropsci2004.0731

Leuchtmann A, Schardl CL, Siegel MR (1994) Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. *Mycologia* **86**, 802–812. doi:10.2307/3760595

Malinowski DP, Belesky DP (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. *Crop Science* **40**, 923–940. doi:10.2135/cropsci2000.404923x

Marks S, Clay K (2007) Low resource availability differentially affects the growth of host grasses infected by fungal endophytes. *International Journal of Plant Sciences* **168**, 1269–1277. doi:10.1086/521834

Martínez-Ghersa MA, Ghersa CM, Benech-Arnold RL, MacDonough R, Sánchez RA (2000) Adaptive traits regulating dormancy and germination of invasive species. *Plant Species Biology* **15**, 127–137. doi:10.1046/j.1442-1984.2000.00033.x

Michel BE (1983) Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiology* **72**, 66–70. doi:10.1104/pp.72.1.66

Morrison DA, Morris EC (2000) Pseudoreplication in experimental designs for the manipulation of seed germination treatments. *Austral Ecology* **25**, 292–296. doi:10.1046/j.1442-9993.2000.01025.x

Neil KL, Tiller RL, Faeth SH (2003) Big sacaton and endophyte-infected Arizona fescue germination under water stress. *Journal of Range Management* **56**, 616–622. doi:10.2307/4003936

R Development Core Team (2010) 'R: a language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna) Available at: http://www.R-project.org

Saikkonen K, Wäli P, Helander M, Faeth SH (2004) Evolution of endophyte-plant symbioses. *Trends in Plant Science* **9**, 275–280. doi:10.1016/j.tplants.2004.04.005

Spiering MJ, Greer DH, Schmid J (2006) Effects of the fungal endophyte, *Neotyphodium lolii*, on net photosynthesis and growth rates of perennial ryegrass (*Lolium perenne*) are independent of in planta endophyte concentration. *Annals of Botany* **98**, 379–387. doi:10.1093/aob/mcl108

Vázquez de Aldana BR, García-Ciudad A, García-Criado B (2008) Interannual variations of above-ground biomass and nutritional quality of Mediterranean grasslands in Western Spain over a 20-year period. *Australian Journal of Agricultural Research* **59**, 769–779. doi:10.1071/AR07359

Vázquez de Aldana BR, Zabalgogeazcoa I, Rubio de Casas R, García Ciudad A, García Criado B (2010) Relationships between the genetic distance of *Epichloë festucae* isolates and the ergovaline and peramine content of their *Festuca rubra* hosts. *Annals of Applied Biology* **156**, 51–61. doi:10.1111/j.1744-7348.2009.00360.x

Wäli PR, Helander M, Saloniemi I, Ahlholm J, Saikkonen K (2009) Variable effects of endophytic fungus on seedling establishment of fine fescues. *Oecologia* **159**, 49–57. doi:10.1007/s00442-008-1202-z

White JF Jr, Torres MS (2010) Is plant endophyte-mediated defensive mutualism the result of oxidative stress protection? *Physiologia Plantarum* **138**, 440–446. doi:10.1111/j.1399-3054.2009.01332.x

Wilkinson HH, Siegel MR, Blankenship JD, Mallory AC, Bush LP, Schardl CL (2000) Contribution of fungal loline alkaloids to protection from aphids in a grass-endophyte mutualism. *Molecular Plant-Microbe Interactions* **13**, 1027–1033. doi:10.1094/MPMI.2000.13.10.1027

Zabalgogeazcoa I, Vázquez de Aldana BR, García Criado B, García Ciudad A (1999) The infection of *Festuca rubra* by the endophyte *Epichloë festucae* in Mediterranean permanent grasslands. *Grass and Forage Science* **54**, 91–95. doi:10.1046/j.1365-2494.1999.00155.x

Zabalgogeazcoa I, García Ciudad A, Vázquez de Aldana BR, García Criado B (2006*a*) Effects of the infection by the fungal endophyte *Epichloë festucae* in the growth and nutrient content of *Festuca rubra. European Journal of Agronomy* **24**, 374–384. doi:10.1016/j.eja.2006.01.003

Zabalgogeazcoa I, Romo M, Keck E, Vázquez de Aldana BR, García Ciudad A, García Criado B (2006*b*) The infection of *Festuca rubra* subsp. *pruinosa* by *Epichloë festucae. Grass and Forage Science* **61**, 71–76. doi:10.1111/j.1365-2494.2006.00509.x

Zabalgogeazcoa I, García Ciudad A, Leuchtmann A, Vázquez de Aldana BR, García Criado B (2008) Effects of choke disease in the grass *Brachypodium phoenicoides. Plant Pathology* **57**, 467–472. doi:10.1111/j.1365-3059.2007.01784.x