Austral Ecology (2006) **31**, 767–775 doi:10.1111/j.1442-9993.2006.01638.x

Effects of the *Neotyphodium* endophyte fungus on dormancy and germination rate of *Lolium multiflorum* seeds

PEDRO E. GUNDEL,[1]* PABLO H. MASEDA,[2] CLAUDIO M. GHERSA[1] AND ROBERTO L. BENECH-ARNOLD[3]
[1]*IFEVA (CONICET), Cátedra de Ecología,* [2]*Cátedra de Fisiología Vegetal and* [3]*Cátedra de Cerealicultura, Facultad de Agronomía, Universidad de Buenos Aires (UBA). Avenue. San Martín 4453, C1417DSE, Buenos Aires, Argentina (Email: gundel@agro.uba.ar)*

Abstract *Neotyphodium* frequently occurs as an endophyte in grasses. Evidence shows enhanced fitness of endophyte infected grasses relative to non-infected ones. Some studies of seed germination show endophyte enhancement of plant fitness in various environments, but inconsistent results indicate that further studies are needed. So far, experiments have failed to separate the confounded effects of population origin and seed management. For this reason, we evaluated the effects of endophyte infection on seed dormancy and germination in *Lolium multiflorum* using an experimental design controlling these factors. Depending on the year of seed production, endophyte infection modified seed response to light quality, affecting predominantly seed dormancy levels. Nevertheless, the endophyte did not affect base temperature or thermal time of germination. We concluded that endophytes were not a strong influence on germination behaviour. We speculate from our results that the presence of the endophyte changes germination by an indirect effect, in extending growth of the maternal plant during seed development and ripening. The direct effect of hyphae in the seed on seed behaviour was disregarded, because the difference between infected and non-infected seed varied within the year of seed production. Future experiments should focus on effects of the endophyte on the canopy of parent plants during seed production and ripening, and, hence, on subsequent dormancy and germination of the seeds.

Key words: dormancy, germination rate, *Lolium multiflorum*, maternal effect, *Neotyphodium*.

INTRODUCTION

Neotyphodium spp. (formerly *Acremonium*) are endophytes that live asymptomatically within many C3 grass species. Evidence shows benefits for both partners in the association, and for this reason it has been considered as a mutualistic relationship (Clay & Schardl 2002). The endophyte benefits the host plant by increasing its tolerance to biotic and abiotic stress (Saikkonen *et al*. 1998; Malinowski & Belesky 2000; Vila-Aiub *et al*. 2003). The plant provides nutrients and protection to the endophyte, and facilitates its propagation because the endophyte is transmitted through the plant seeds (vertical transmission) (Latch *et al.* 1987; Clay 1990; Ravel *et al*. 1997). This mutualism is asymmetric, since the association is obligate for the endophyte, but it is facultative for the plant (Leuchmann 1997).

A high frequency of infection in many grass populations is commonly associated with the suggestion that the endophyte improves the plant's fitness. Germination is an aspect of plant fitness and could be affected by endophytes, but information on this topic is scarce and ambiguous. It has been found that endophyte infection increases germination in *Festuca arundinacea* (Pinkerton *et al*. 1990), *Lolium perenne* (Clay 1987) and *Bromus setifolius* (Novas *et al*. 2003). However, some studies have found that the endophyte did not affect seed germination of *Festuca arizonica* (Neil *et al*. 2003; Faeth *et al*. 2004) or *F. arundinacea* (Bacon 1993). It can be argued that these inconsistencies arise from comparing experiments in which endophyte effects were confounded with both the genetic origin of the host plants, and also with conditions of seed production and after-ripening. Seed phenotypic response is controlled by genotype and prevailing environmental conditions during seed development and seed after-ripening (Bewley & Black 1982; Gutterman 2000).

Germination and dormancy of seeds are both integral processes in the emergence patterns of seedlings (Benech-Arnold *et al*. 2000). On the one hand, the thermal time model, which assumes a linear relationship between germination rate and incubation temperature for a seed population, has been used to characterize germination at suboptimal temperatures of non-dormant seeds in many species (García-Huidobro *et al*. 1982; Gummerson 1986). This model function is determined by two parameters: Base

*Corresponding author.
Accepted for publication February 2006.

temperature and Thermal time (for definitions see Methods). On the other hand, some forms of seed dormancy allow seeds to sense the environment (Casal & Sanchez 1998) and are difficult to characterize (Benech-Arnold *et al.* 2000). For example, seed behaviour under plant canopies is controlled, in some species, through changes in light quality and these responses are mediated by phytochromes. Germination promotion by red light (R) relative to germination in darkness is known as LFR (low-fluence response) (Casal *et al.* 1998), and is reversible by different R (red)/FR (far red) ratios. In many cases, seed responses to light depend on the temperature conditions (Pons 2000).

Lolium multiflorum Lam. is a C3 grass species naturalized in temperate subhumid grasslands of the Pampa Region, Argentina (Soriano *et al.* 1991), and is often infected with the seed-transmitted fungal endophyte *Neotyphodium occultans* (Moon *et al.* 2000; Christensen *et al.* 2002). After-ripening, the seeds present primary dormancy, which is lost by the effect of low winter temperatures (Rodriguez *et al.* 1998). When low temperatures have broken dormancy, germination is promoted by high levels of red (R)/far-red (FR) light ratio and by alternating temperatures (Deregibus *et al.* 1994). Although there are studies indicating that infection of seed by the *Neotyphodium* endophyte affects germination and seedling emergence in *L. multiflorum* (Vila-Aiub *et al.* 2005), there is no evidence of how dormancy and thermal time model parameters are affected by endophyte infection of seeds.

Endophytes could affect seed germination through two mechanisms: (i) a direct effect of the fungal hyphae in the seeds during germination; or (ii) an indirect effect of the fungus through modifying the plant's interaction with environmental conditions during its growth and development (i.e. maternal effect). The first mechanism is inferred because the amount of hyphae per plant biomass unit is very high in seeds (White & Cole 1985). The second mechanism is inferred through evidence showing the effects of fungal endophytes on growth and development of host plants (Clay & Schardl 2002; Faeth *et al.* 2004; Vila-Aiub *et al.* 2005). In this work, we evaluated the effect of a wide range of thermal and light quality conditions on dormancy and germination rate in *L. multiflorum* seeds infected or non-infected with *Neotyphodium* endophytes in controlled and natural environments.

METHODS

Seed origin

Lolium multiflorum seeds were collected from old-field communities of the Inland Pampa region (Carlos Casares, Argentina, 34°06′S, 60°25′W). *Neotyphodium* endophyte infection (E^+) in this population is >85% (Vila-Aiub *et al.* 2005). To generate the non-infected biotype of *L. multiflorum* (E^-), collected seeds were treated with Triadimenol (Beta-(4-chlorophenoxy)-alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol) fungicide (150 g a.i. kg^{-1}). To obtain seeds of E^- and E^+ biotypes, treated and untreated seeds were sown in plots in an experimental field at the School of Agriculture, Facultad de Agronomía, Universidad de Buenos Aires (34°35′S, 58°35′W) free of other *L. multiflorum* seeds. E^- seedlings were sprayed with a Benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) fungicide (500 g a.i. kg^{-1}) when they had 2–3 leaves. At the end of the growing cycle all seeds from both biotypes were harvested. Dry seeds of each biotype were stored inside glass jars at 10°C in darkness. Both biotypes were re-sown annually for four successive years, allowing for cross pollination between biotypes. Endophyte infection in each plot was followed by collecting seed samples and examining them through a light microscope (Latch *et al.* 1987).

Germination experiments

Laboratory

The effects of temperature and light quality on germination of seed of *L. multiflorum* infected and non-infected with *Neotyphodium* were studied by means of two experiments carried out in growth chambers. In the first experiment, seeds were produced in year 2002 and allowed to ripen in storage for 4 months. Endophyte infection was >85% for (E^+) and <6% for (E^-). For the second experiment, seeds were produced in year 2003 and allowed to ripen in storage for 5 months, and endophyte infection was >90% for (E^+) and <10% (E^-). Groups of 30 seeds of each biotype were sown on filter papers in 36 and 45 Petri dishes for the first and second experiment, respectively. Distilled water (6 mL) was added and the Petri dishes were incubated at 20°C for 4 h in a growth chamber. After that, they were separated into three groups and exposed to different light conditions: (a) 20-minute red light pulse (R, 660 nm, 30 μmol $m^{-2}s^{-1}$); (b) 5-minute far red pulse (FR, 730 nm, 17 μmol m^{-2} s^{-1}); and (c) continuous darkness (D). All Petri dishes were placed under the same source of R or FR light. Finally, three Petri dishes with E^+ seeds and three with E^- seeds from each light treatment were incubated at 10°C, 20°C and 25°C continuous or 20/30°C alternating (12 h AM/12 h PM) temperatures. In the second experiment a 5°C continuous temperature was added to the treatments. In order to reduce the non-independent relationship among Petri dishes (Morrison & Morris

2000) they were randomly distributed into each growth chamber. During the whole experimental period, the growth chambers were maintained in darkness, and Petri dishes were covered with a black plastic film to prevent light from reaching the seeds. Germination (visible radicle protrusion) was recorded periodically in the dark room with a safe green light. When germination reached a plateau, the seeds remaining in each Petri dish were exposed to the natural light and incubated at 10°C until dormancy release and germination occurred.

Field

To test the ecological significance of the mechanisms found in the laboratory experiments a field experimental set-up was installed upon a homogeneous and closed canopy of *Bromus catharticus* Vahl. We generated three different canopy levels: (a) open canopy (clipped down to the soil surface); (b) medium canopy (30 cm height); and (c) closed canopy (90 cm height). To produce different temperature regimes, half of these plots (one per canopy treatment) were covered with a 50% shading black net generating shaded and non-shaded treatments. As a result, six plots (60 cm wide and 150 cm long) were obtained from the combination of canopy and shaded treatment.

In order to characterize the light environment, radiation flux of red (R, 660 nm) and far red (FR, 730 nm) light and R/FR ratio were measured using a radiometer sensor (SKR 110, Skye Instruments, Powys, UK). During the whole experiment, minimum, maximum and mean temperatures were recorded daily on each treatment using thermocouples placed on the soil surface connected to a micrologger (Model 21X, Campbell Scientific, Inc., Logan, UT) (Fig. 1).

Thirty seeds produced in 2003 from each biotype and stored for 7 months since harvesting were sown in plastic boxes ($10 \times 15 \times 5$ cm) on a cotton layer (0.5 cm) covered by a filter paper. All boxes were watered once, at the beginning of the experiment, with 45 mL of distilled water. In each canopy and shaded treatment, five boxes were randomly arranged. During the whole experiment, the boxes were periodically (2–3 days) and, at night time, transferred inside a black box to a dark room where the seed germination was recorded under safe green light. When germination percentage reached a plateau, remaining seeds were exposed for 20 min to a red light pulse and incubated at 10°C to discard dead from live seeds.

Data analysis

Results of both laboratory experiments (2002 and 2003) were analysed separately by means of ANOVA testing the effects of: biotypes (E^+ and E^-), temperatures and light treatments on the final percentage of germination. Each Petri dish was considered as a pseudo-replication due to the possible confounded effects associated with using only a single chamber for each temperature treatment (Hurlbert 1984; Morrison & Morris 2000). In the field experiment, repeated-measures ANOVA were used to test the effects of biotype, canopy and shading treatments, and time. In the same way, each plastic box was considered as a pseudo-replication because there was only a single field plot per canopy and shaded treatment. In all experiments the values of proportion germinating were arcsine $\sqrt{x}$ transformed to comply with the assumption of homogeneity of variance in the analysis of variance. All tests were performed using Statistica software (StatSoft, Inc. 1984–2001).

The thermal time model was used to test the germination rate of non-dormant seeds coming from the red light treatment of the laboratory experiments of both biotypes in relation to incubation temperature (Gummerson 1986). According to this, the base temperature below which germination does not occur (T_b)

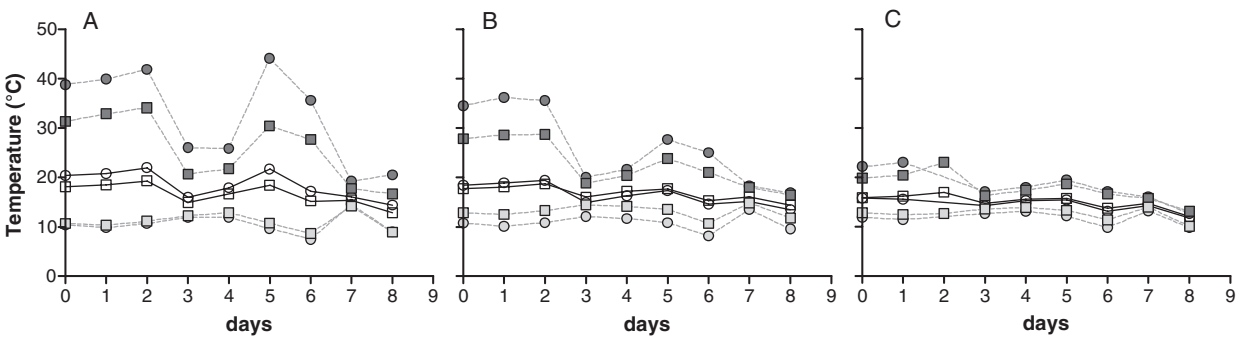

Fig. 1. Temperature dynamics (mean temperature in day: white symbol, maximum value in day: black symbol and minimum value in day: grey symbol) under different environmental conditions: (A) open canopy; (B) medium canopy; and (C) closed canopy; and shaded treatment: non-shaded (circles) and shaded (squares) during the field experiment.

was assumed to be constant among different seed fractions (Bradford 2002). The thermal time required depends on the considered seed fraction (Benech-Arnold *et al.* 1990), and is the accumulated temperature above the T_b to reach a particular percentage of seed germination ($\theta_T(g)$). To adjust this model, Gompertz functions describing the dynamics of accumulated percentage of germination in relation to time (hours) (Tipton 1984) were fitted for each Petri dish to estimate the number of hours to 50% germination (t_{50}). Germination rates were obtained as $1/t_{50}$. Linear regressions relating germination rates with incubation temperatures were fitted (Eqn 1). The T_b was obtained by linear extrapolation until germination rate was zero (*x*-intercept). The $\theta_T(50)$ was estimated as the inverse of the slope of the linear regression, representing the accumulated thermal units per hour to reach 50% of germination. The relationship between germination rate and temperature is characterized by the following equation:

$$GR_g = 1/t_g = (T - T_b)/\theta_T(g) \quad (1)$$

Where GR_g is germination rate (in our case: 1/time to reach 50% of germination), T is the incubation constant temperature (here: 5°C, 10°C, 20°C, and 25°C), T_b is the base temperature, and $\theta_T(50)$ is the thermal time to complete 50% of germination (for more details see: Bradford 1995). The goodness of the fitted models was determined by means of a 'Runs test', and differences between biotypes were tested by slope and *y*-intercept comparison (Motulsky & Christopoulos 2003).

RESULTS

Laboratory experiments

While the biotype effect on seed germination varied between years, temperature and light treatments had significant effects in both years (Figs 2,3). In the 2002 experiment, high temperatures inhibited seed germination independently of the light treatment ($P = 0.559$) (Fig. 2A,B) and of the endophyte infection level ($P = 0.842$) (Table 1). Alternating temperatures slightly promoted seed germination, having a strongest effect on E^+ seeds within red light and darkness treatments, but that effect could not bypass the inhibitory effect of far red light treatment (Fig. 3A).

In the 2003 experiment, seed germination was affected by light quality depending on endophyte infection status ($P = 0.045$) (Table 1, Fig. 2C,D). Although the ANOVA did not detect an interaction between light and temperature ($P = 0.174$), the inhibitory effect of high temperatures observed in the previous year was partially bypassed for the red light treatment (Fig. 2C,D). The general dormancy level of seeds was higher in the E^+ biotype than in the E^- biotype (Fig. 2C,D). Alternating temperatures did

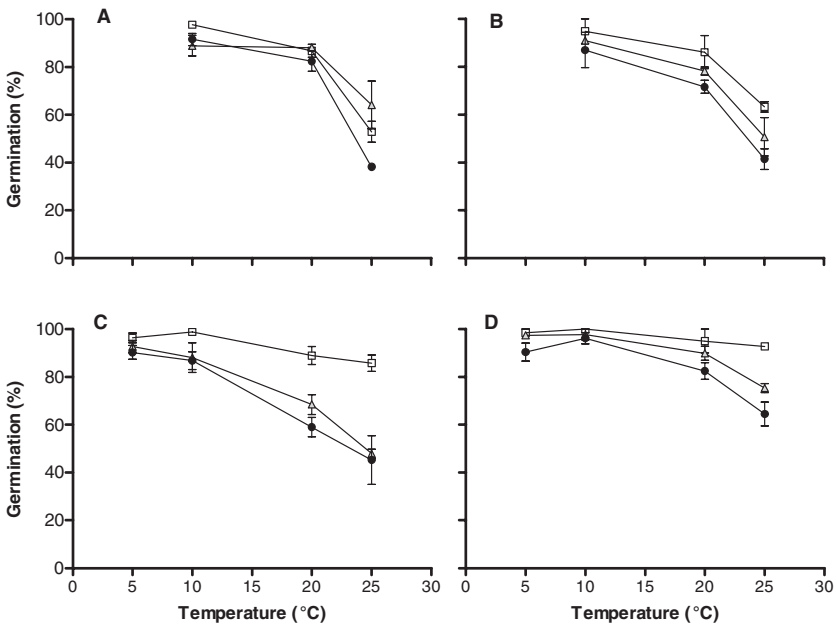

Fig. 2. Germination of *Lolium multiflorum* infected (E^+) and non-infected (E^-) seeds in a temperature incubation gradient (A and B: 10°C, 20°C and 25°C; C and D: 5°C, 10°C, 20°C and 25°C) and different light conditions: red pulse (squares), darkness (triangles) and far red pulse (circles). A: E^+, 2002; B: E^-, 2002; C: E^+, 2003; D: E^-, 2003. Each point is the average of $n = 3$ (30 seeds each) ±s.e.

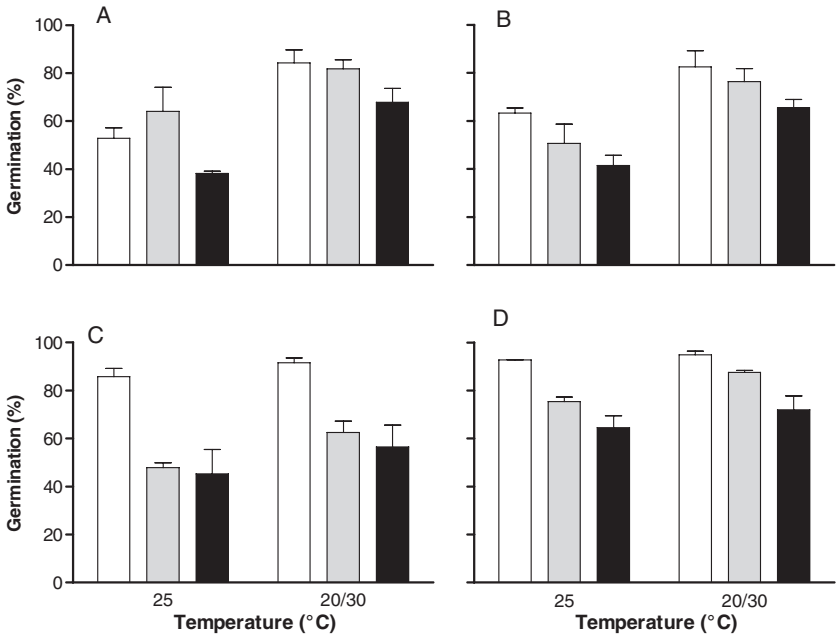

Fig. 3. Germination of *Lolium multiflorum* infected (E^+) and non-infected (E^-) seeds in relation to incubation temperature (25°C constant and 20°C/30°C alternating temperatures) and different light conditions: red pulse (white bar), darkness (grey bar) and far red pulse (black bar). A: E^+, 2002; B: E^-, 2002; C: E^+, 2003; D: E^-, 2003. Each point is the average of $n = 3$ (30 seeds each) ±s.e.

Table 1. Statistical results of three-way ANOVA of seed germination of each laboratory experiment (2002 & 2003). Data were angular transformed by arcsine $\sqrt{x}$

Source of variation	d.f.	MS	*F*-value	*P*
Year 2002				
Biotype	1	0.020	1.10	0.299
Temperature	3	0.830	55.34	**<0.001**
Light	2	0.200	13.39	**<0.001**
Biotype × temperature	3	0.004	0.28	0.842
Biotype × light	2	0.020	1.32	0.278
Temperature × light	6	0.010	0.84	0.559
Biotype × temperature × light	6	0.010	0.56	0.768
Year 2003				
Biotype	1	0.680	56.11	**<0.001**
Temperature	4	0.550	45.34	**<0.001**
Light	2	0.750	62.42	**<0.001**
Biotype × temperature	4	0.020	1.45	0.228
Biotype × light	2	0.040	3.27	**0.045**
Temperature × light	8	0.020	1.51	0.174
Biotype × temperature × light	8	0.004	0.32	0.955

d.f., degrees of freedom; MS, mean squares.

not promote E^+ seed germination at all light treatments as observed for 2002 seeds (Fig. 3C,D).

Development of a thermal time model

Linear regressions relating germination rate and constant temperature of incubation for each biotype and year did not differ in either slope and in *y*-intercept (Data not shown). For this reason, all data were pooled and a single model was fitted (Fig. 4). The thermal time required for 50% of germination was 909.09°C h and the base temperature was −1.71°C.

Field experiment

Canopy and shade treatments modified the thermal regimes through modifications in mean, maximum and minimum temperature values (Fig. 1). In the

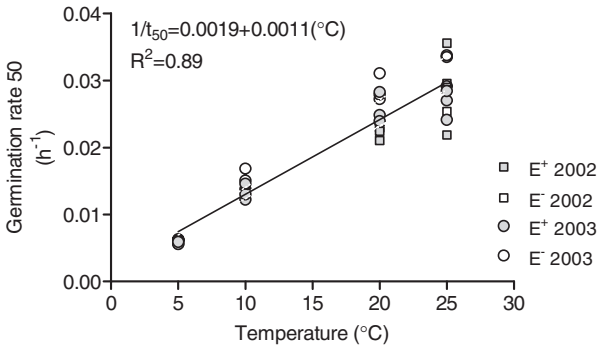

Fig. 4. Germination rate 50 defined as 1/time to reach 50% of germination ($1/t_{50}$) of *Lolium multiflorum* non-dormant seed fraction in relation to incubation temperature. The regression model includes only seed from red light pulse treatment at all incubation constant temperatures of years (2002 and 2003), infection status (E^+ & E^- biotypes) and three pseudo-replications. All data had a close fit with this linear regression (Runs test, $P = 0.11$).

same way, treatments modified effectively the light quality environment (Table 2).

Canopy and shaded treatments affected the final germination percentage ($P < 0.001$) independently of the biotype ($P = 0.755$) (Table 3). Repeated-measures ANOVA detected a three–way interaction among canopy, biotype and time ($P < 0.001$). In spite of this, the main difference in seed germination was caused by the environmental conditions rather than the biotypes (Fig. 5).

DISCUSSION

In this work, the origin of E^- and E^+ biotypes, taken from the same wild population of *L. multiflorum*, and both time and environmental conditions for seed ripening and after-ripening were controlled, since seeds of both biotypes came from plants grown in a common garden and stored in the same way. This can rule out

Table 2. Radiation flux (μmol s^{-1} m^{-2}) of red light (R, 660 nm), far red light (FR, 730 nm) and red/far red ratio (R/FR) in: open, medium and closed canopy; under non-shaded and shaded conditions. Each value is the average of four measurements within each treatment done at the start of the experiment.

	Non shaded			Shaded		
	R (s.e.)	FR (s.e.)	R/FR (s.e.)	R (s.e.)	FR (s.e.)	R/FR (s.e.)
Open canopy	1524.7 (33.8)	1374.5 (35.0)	1.10 (0.0)	627.7 (51.7)	543.2 (37.9)	1.10 (0.0)
Medium canopy	164.2 (46.0)	301.5 (39.0)	0.49 (0.0)	91.7 (43.4)	183.5 (39.2)	0.40 (0.0)
Closed canopy	22.7 (8.4)	58.5 (24.9)	0.16 (0.0)	5.5 (0.9)	53.0 (1.4)	0.11 (0.0)

s.e., standard error.

Table 3. Statistical results of repeated-measures ANOVA of seed germination of field experiment (2003). Data were angular transformed by arcsine $\sqrt{x}$

Source of variation	d.f.	MS	*F*-value	*P*
Biotype	1	0.003	0.135	0.714
Canopy	2	0.426	16.458	**<0.001**
Shaded	1	0.115	4.428	**0.040**
Biotype × canopy	2	0.001	0.033	0.967
Biotype × shaded	1	0.036	1.424	0.238
Canopy × shaded	2	0.637	24.589	**<0.001**
Biotype × canopy × shaded	2	0.007	0.282	0.755
Time	2	4.052	818.895	**<0.001**
Time × biotype	2	0.002	0.421	0.657
Time × canopy	4	0.660	133.493	**<0.001**
Time × shaded	2	0.044	8.937	**<0.001**
Time × biotype × canopy	4	0.037	7.580	**<0.001**
Time × biotype × shaded	2	0.004	0.821	0.443
Time × canopy × shaded	4	0.038	7.836	**<0.001**
Time × biotype × canopy × shaded	4	0.008	1.625	0.174

d.f., degrees of freedom; MS, mean squares.

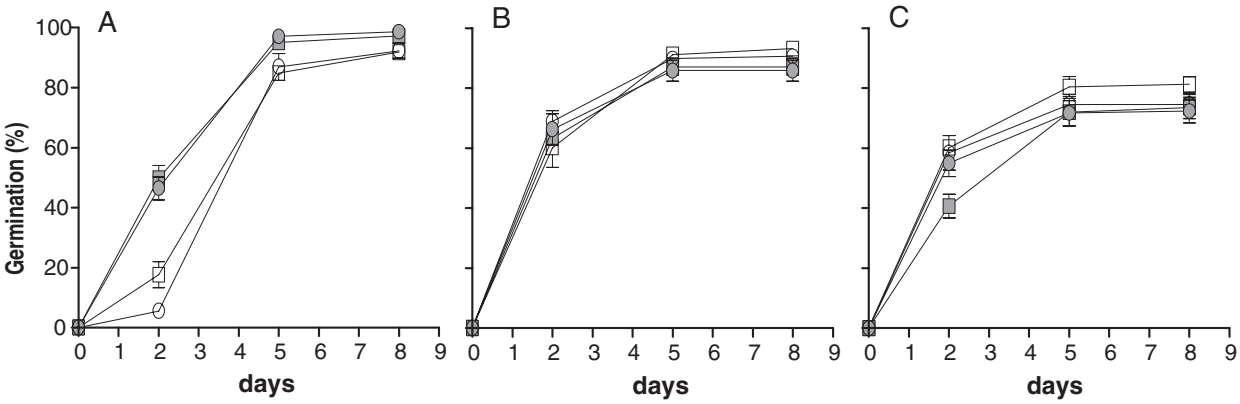

Fig. 5. Germination of *Lolium multiflorum* infected (E^+) (squares) and non-infected (E^-) (circles) seeds under different environmental conditions: open canopy (A), medium canopy (B) and closed canopy (C); non-shaded (white symbols) and shaded (grey symbols). Each point is the average of $n = 5$ (30 seeds each) ±s.e.

the possible effects of the environment in determining different seed phenotypes. This improved the experimental procedures compared to previous studies in which the effects of endophytes on host germination may have been confounded with the population origin. In those previous studies reported differences could have stemmed from either the genetic background or the environment.

Results presented here show that the *Neotyphodium* endophyte had a small effect on the germination behaviour of *L. multiflorum* seeds. The laboratory experiments showed that the effect of the endophyte on the germination behaviour of *L. multiflorum* seeds depended on the environmental conditions. For example, when we incubated the seeds at 10°C there were no endophyte effects on dormancy, but at higher incubation temperatures the endophyte reduced the percentage of germination. The endophyte effect in these conditions was predominantly via increases in seed dormancy levels (Figs 2,3) and not in changes of germination rate (Fig. 4). As *L. multiflorum* seeds require cold temperatures for dormancy release (Rodriguez *et al.* 1998), the low 10°C temperature did not allow expression of seed dormancy, and endophyte effects became unimportant. However endophyte effects on dormancy occurred only in one year, suggesting that the impact of endophytes on seed dormancy varies depending on the environment under which the seeds had developed. This interannual variability, affecting the requirements for dormancy release, allowed us to infer that the 'direct effect' of the endophyte on germination behaviour is less important than previously suspected.

The interannual variability strongly suggested that endophytes may have affected germination responses indirectly. We speculate that the more extended growing period of endophyte-infected relative to non-infected plants may have altered the growing conditions during seed development, and that this environmental difference may be an important variable depending on the year. Previous studies have shown that the flowering phase in the E^+ *L. multiflorum* biotype is longer than in the E^- biotype and plants retain green tissues for longer time (Vila-Aiub *et al.* 2005), but this difference may be reduced or amplified by drought, wind, or shifts in temperatures. It is also well documented that changes in the environmental conditions of a plant canopy affect seed traits in relation to the response of seed germination to light quality (Gutterman 2000). This has been interpreted in terms of Pr – Pfr equilibrium modulated by the R/FR ratio (Casal & Sanchez 1998). For instance, seeds in which embryos are retained within green tissues for prolonged periods will have most of the phytocrome in the Pr form (Cresswell & Grime 1981). Also, the Pfr – Pr equilibrium is affected by temperature during seed maturity, which is also controlled by leaf area duration.

Regarding the germination rate of the non-dormant seed fraction, the base temperature (T_b) is expected to be a genotypic trait (Squire *et al.* 1997), although the environment in which seeds are produced could cause variations in this parameter (Cheng & Bradford 1999). A possible hypothesis was that the endophyte could affect T_b and $\theta_T(50)$, due to its effects on growth and development in vegetative and reproductive stages of mother plants (Vila-Aiub *et al.* 2005). However, results presented here suggested that the endophyte affected neither T_b nor $\theta_T(50)$ in any of the experimental conditions explored. For annual species, earlier emergence and the capacity to establish a large number of individuals are important attributes in determining the competitive ability (Harper 1977). The capacity of the seed to sense accurately the environmental stimuli are important for this reason (Benech-Arnold *et al.* 2000). An overview of the results,

suggested that the impact of the endophyte was associated with this latter capacity (i.e. red light requirements for breaking dormancy) rather than its effect on germination rate. Dormancy is a risk-avoiding feature that will be favoured, and selected, in most ecological scenarios (Martínez-Ghersa *et al.* 2000). Due to the higher dormancy level and specific light requirements of the infected seeds to germinate, this characteristic probably contributes to enhanced seedling establishment in the field.

In the field experiment, we confirmed that endophytes did not appear to have a strong influence on germination behaviour of *L. multiflorum* seeds. The differences in germination percentage were attributed mainly to effects of environmental variables. These included a reduction in germination with a decrease in R/Fr ratio (Fig. 5) and also with high maximum temperatures (Fig. 5, day 2). Despite the small impact that the endophyte had on *L. multiflorum* seed ecophysiological behaviour, it is important to accept that these effects could improve fitness of infected individual in particular ecological conditions which were not tested in this experiment. For this reason, it is important to encourage experiments that analyse the effect of the endophyte, manipulating the environment in serial phases of development in order to establish which ones are the most affected by this fungus infection.

ACKNOWLEDGEMENTS

The work was funded by grant UBACYT G042. PEG holds a scholarship from National Research Council, Argentina. We thank M. Omacini, D. Batlla and R. Fernández for their comments on the paper.

REFERENCES

Bacon C. W. (1993) Abiotic stress tolerances (moisture, nutrients) and photosynthesis in endophyte-infected tall fescue. *Agric. Ecosyst. Environ.* **44,** 123–41.

Benech-Arnold R. L., Ghersa C. M., Sánchez R. A. & Insausti P. (1990) Temperature effects on dormancy release and germination rate in *Sorghum halepense* (L.) Pers. seeds: A quantitative analysis. *Weed Research* **30,** 81–9.

Benech-Arnold R. L., Sánchez R. A., Forcella F., Kruk B. C. & Ghersa C. M. (2000) Environmental control of dormancy in weed seed soil banks. *Field Crops Res.* **67,** 105–22.

Bewley J. D. & Black M. (1982) *Physiology and Biochemistry of Seeds in Relation to Germination*. Springer Verlag, Berlin.

Bradford K. J. (1995) Water relations in seed germination. In: *Seed Development and Germination* (eds J. Kigel & G. Galili) pp. 351–96. Marcel Dekker, Inc., New York.

Bradford K. J. (2002) Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* **50,** 248–60.

Casal J. J. & Sanchez R. A. (1998) Phytochromes and seed germination. *Seed Sci. Res.* **8,** 317–29.

Casal J. J., Sanchez R. A. & Botto J. F. (1998) Modes of action of phytochromes. *J. Exp. Bot.* **49,** 127–38.

Cheng Z. & Bradford K. J. (1999) Hydrothermal time of tomato seed germination responses to priming treatment. *J. Exp. Bot.* **50,** 89–99.

Christensen M. J., Bennett R. J. & Schmid J. (2002) Growth of *Epichloë/Neotyphodium* and p-endophytes in leaves of *Lolium* and *Festuca* grasses. *Mycol. Res.* **106,** 93–106.

Clay K. (1987) Effects of fungal endophytes on the seed and seedling biology of *Lolium perenne* and *Festuca arundinacea*. *Oecologia* **73,** 358–62.

Clay K. (1990) Fungal endophytes of grasses. *Annu. Rev. Ecol. Syst.* **21,** 275–9.

Clay K. & Schardl C. (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am. Nat.* **160,** 99–127.

Cresswell E. G. & Grime J. P. (1981) Induction of a light requirement during seed development and its ecological consequences. *Nature* **291,** 583–5.

Deregibus V. A., Casal J. J., Jacobo E. J., Gibson D., Kauffman M. & Rodriguez A. M. (1994) Evidence that heavy grazing may promote the germination of *Lolium multiflorum* seeds via phytochrome-mediated perception of high red/far-red ratios. *Funct. Ecol.* **8,** 536–42.

Faeth S. H., Helander M. L. & Saikkonen K. T. (2004) Asexual *Neotyphodium* endophytes in a native grass reduce competitive abilities. *Ecol. Lett.* **7,** 304–13.

García-Huidobro J., Monteith J. L. & Squire G. R. (1982) Time, temperature and germination of pearl millet (*Pennisetum thyphoides* S. & H.). I. Constant temperatures. *J. Exp. Bot.* **33,** 288–96.

Gummerson R. J. (1986) The effect of constant temperatures and osmotic potential on the germination of sugar beet. *J. Exp. Bot.* **37,** 729–41.

Gutterman Y. (2000) Maternal effects on seeds during development. In: *Seeds. The Ecology of the Regeneration in Plant Communities*, 2nd edn (ed. M. Fenner) pp. 59–84. CAB International, London.

Harper J. L. (1977) *Population Biology of Plants*. Academic Press, London.

Hurlbert S. H. (1984) Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* **54,** 187–211.

Latch G. C. M., Potter L. R. & Tyler B. F. (1987) Incidence of endophytes in seeds from collections of *Lolium* and *Festuca* species. *Ann. Appl. Biol.* **111,** 59–64.

Leuchmann A. (1997) Ecological diversity in *Neotyphodium*-infected grasses as influenced by host and fungus characteristics. In: *Neotyphodium/Grass Interactions* (eds C. W. Bacon & N. S. Hill) pp. 93–108. Plenum Press, New York and London.

Malinowski D. P. & Belesky D. P. (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. *Crop Sci.* **40,** 923–40.

Martínez-Ghersa M. A., Ghersa C. M., Benech-Arnold R. L., MacDonough R. & Sánchez R. A. (2000) Adaptive traits regulating dormancy and germination of invasive species. *Plant Species Biol.* **15,** 127–37.

Moon C. D., Scott D. B., Schardl C. L. & Chistensen M. J. (2000) The evolutionary origins of *Epichloe* endophytes from annual ryegrasses. *Mycologia* **92,** 1103–18.

Morrison D. A. & Morris E. C. (2000) Pseudoreplication in experimental designs for the manipulation of seed germination treatments. *Austral Ecol.* **25,** 292–6.

Motulsky H. J. & Christopoulos A. (2003) *Fitting Models to Biological Data Using Linear and Nonlinear Regression: A Practical Guide to Curve Fitting*. GraphPad Software Inc., San Diego. Available from URL: http://www.graphpad.com

Neil K. L., Tiller R. L. & Faeth S. H. (2003) Big sacaton and endophyte-infected Arizona fescue germination under water stress. *J. Range Manage.* **56,** 616–22.

Novas M. V., Gentile A. & Cabral D. (2003) Comparative study of growth parameters on diaspores and seedlings between populations of *Bromus setifolius* from Patagonia, differing in *Neotyphodium* endophyte infection. *Flora* **198,** 421–6.

Pinkerton B. W., Rice J. S. & Undersander D. J. (1990) Germination in *Festuca arundinacea* as affected by the fungal endophyte, *Acremonium coenophialum*. In: *Proceedings of the International Symposium: On Acremonium/Grass Interactions* (eds S. S. Quinsenberry & R. E. Joost) p. 176, New Orleans.

Pons T. L. (2000) Seed responses to light. Seeds. In: *The Ecology of the Regeneration in Plant Communities*, 2nd edn (ed. M. Fenner) pp. 237–60. CAB International, London.

Ravel C., Michalakis Y. & Charmet G. (1997) The effect of imperfect transmission on the frequency of mutualistic seed-borne endophytes in natural populations of grasses. *Oikos* **80,** 18–24.

Rodriguez A. M., Jacobo E. J. & Deregibus V. A. (1998) Germination behaviour of italian ryegrass in flooding pampa rangelands. *Seed Sci. Res.* **8,** 521–8.

Saikkonen K., Faeth S. H., Helander M. & Sullivan T. J. (1998) Fungal endophytes: a continuum of Interactions with Host Plants. *Annu. Rev. Ecol. Syst.* **29,** 319–43.

Soriano A., León R. J. C., Sala O. E. *et al.* (1991) Río de la Plata grasslands. In: *Ecosystems of the World 8A. Natural Grasslands. Introduction and Western Hemisphere* (ed. R. T. Coupland) pp. 367–407. Elsevier, New York.

Squire G., Marshall B., Dunlop G. & Wright G. (1997) Genetic basis of rate-temperature characteristics for germination in oilseed rape. *J. Exp. Bot.* **48,** 869–75.

Tipton J. L. (1984) Evaluation of three growth curve models for germination data analysis. *J. Am. Soc. Hortic. Sci.* **109,** 451–4.

Vila-Aiub M. M., Gundel P. E. & Ghersa C. M. (2005) Fungal endophyte infection changes growth attributes in Lolium multiflorum Lam. *Austral Ecol.* **30,** 49–57.

Vila-Aiub M. M., Martínez-Ghersa M. A. & Ghersa C. M. (2003) Evolution of herbicide resistance in weeds: vertically transmitted fungal endophytes as genetic entities. *Evol. Ecol.* **17,** 441–56.

White J. F. Jr & Cole G. T. (1985) Endophyte–host association in forage grasses. I. Distribution of fungal endophytes in some species of Lolium and Festuca. *Mycologia* **77,** 323–7.