

Vázquez-de-Aldana B.R., Romo M., García-Ciudad A., Petisco C. and García-Criado B.

TITULO: Effects of fungal endophyte infection in the grass *Festuca rubra* on germination and growth of four legume species

LIBRO: Grassland in a changing world Eds. H. Schnyder, J. Isselstein, F. Taube, J. Schellberg, M. Wachendorf, A. Herrmann, M. Gierus, K. Auerswald, N. Wrage, A. Hopkins. EGF, Universität Göttingen
Grassland Science in Europe Vol 15., pp- 368-370



EGF 2010
Kiel Germany

Grassland in a changing world

Edited by

**H. Schnyder
J. Isselstein
F. Taube
K. Auerswald
J. Schellberg
M. Wachendorf
A. Herrmann
M. Gierus
N. Wrage
A. Hopkins**



**VOLUME 15
GRASSLAND SCIENCE IN EUROPE**

Effects of fungal endophyte infection in the grass *Festuca rubra* on germination and growth of four legume species

Vázquez-de-Aldana B.R., Romo M., García-Ciudad A., Petisco C. and García-Criado B.
Instituto de Recursos Naturales y Agrobiología de Salamanca, IRNASA-CSIC; Apdo 257; 37071 Salamanca; Spain

Corresponding author: beatriz.dealdana@irnasa.csic.es

Abstract

Red fescue (*Festuca rubra*) is a perennial grass present in a wide range of ecological conditions. It is also included in mixtures with other grasses and legumes for several purposes. The objective of this paper was to determine the effect of *Festuca rubra* plants, infected and non-infected by the endophyte *Epichloë festucae*, on the germination and seedling growth of four legume species. During three weeks, a greenhouse experiment was carried out with infected and non-infected plants of *F. rubra*, and four legume species: *Trifolium pratense*, *Trifolium repens*, *Trifolium subterraneum* and *Lotus corniculatus*. The emergence of the legumes was not affected by the presence of *F. rubra* plants. However, the length and biomass production of the four legumes was reduced in the presence of *F. rubra* plants. The decrease in shoot length was greater than that in root length. There was a significant effect of endophyte infection status of *F. rubra* plants on the root length and root biomass of legumes. The reduction in root length and root dry weight of legume seedlings was greater in the presence of infected plants than in the presence of non-infected plants.

Keywords: red fescue, endophytes, *Epichloë*, competition, legumes

Introduction

Festuca rubra is a perennial grass, very persistent and tolerant of a wide range of ecological conditions. Compared with other grass species, it grows well on soils of poor to moderate fertility (Peeters, 2004). It is frequent in natural grasslands with complex floristic composition, and it is included in mixtures for dry soils, mountain areas and extensive use with other grasses and with legumes (Peeters, 2004). In companion with legumes it is also used for silage making (Laser and Von Boberfeld, 2004) and in post-fire recovery (Fernández-Abascal *et al.*, 2003).

In Mediterranean grasslands of western Spain, a high percentage of *F. rubra* plants are asymptotically infected by the fungal endophyte *Epichloë festucae* (Zabalgogea *et al.*, 1999). A number of studies indicate that endophyte-infected grasses exhibit a considerable competitive advantage over their non-endophytic congeners. Thus, as compared to non-infected, infected plants are more resistant to abiotic stress factors such as drought, heavy metal accumulations and nutrient deficiency (Kuldau and Bacon, 2008), as well as more resistant to invertebrate herbivores due to the alkaloid production. This may affect the growth of companion species in mixtures, for instance, *Trifolium pratense* performance appeared to be better in the presence of endophyte-free *Festuca arundinacea* plants (Malinowski *et al.*, 1999).

The objective of the research reported in this paper was to determine the effect of *F. rubra* plants, infected and non-infected by *E. festucae* endophyte, on the germination and seedling growth of four legume species (*Trifolium pratense*, *Trifolium repens*, *Trifolium subterraneum* and *Lotus corniculatus*).

Materials and methods

Festuca rubra plants infected by *E. festucae* endophyte were collected from ‘dehesa’ grasslands in western Spain (province of Salamanca). Infection by *E. festucae* endophyte was verified by microscopic analysis of stem-pith scraping, as well as by isolation of the fungus from stems and leaf sheaths on potato dextrose agar. Plants were grown in pots until they were divided into several ramets; one half of the ramets was used to produce endophyte-free tillers by treatment with propiconazole (TILT®, Ciba), a systemic fungicide. In this way, we obtained infected (E+) and non-infected (E-) plants derived from a common mother plant (half-sib lines). All plants were transplanted in a research farm. Seeds produced were collected and E+ and E- seeds were obtained from ramets of each original plant. Seeds of the legumes *T. pratense*, *T. repens*, *T. subterraneum* and *L. corniculatus* were obtained from Zulueta Co.

Seeds of red fescue (E+ and E-) were germinated and individual plants were grown in pots in a mixture containing peat moss, perlite and sand, in a glasshouse. After six months, plants of equal size were selected and individually transplanted into new pots and placed in a growth chamber with a photoperiod 16 h light at 25 °C and 8 h dark at 20 °C during two acclimatization weeks. The experiment consisted of a factorial combination of four legume species and two infection levels with ten replications, and it was carried out in pots containing one plant of *F. rubra* and 10 seeds of the legume species. As control, ten seeds of the legume were sown in pots without *F. rubra* plants. Seedling emergence was controlled daily for three weeks, until no further seedlings emerged. After 21 days, seedlings were harvested; shoot and root lengths were obtained from scanned images and shoot and root biomass were obtained from dried samples. Data were presented as percentage of reduction over control based on mean values for all ten seedlings per pot. For each legume species, analysis of variance with endophyte infection as factor was applied to the data (SPSS v17.0).

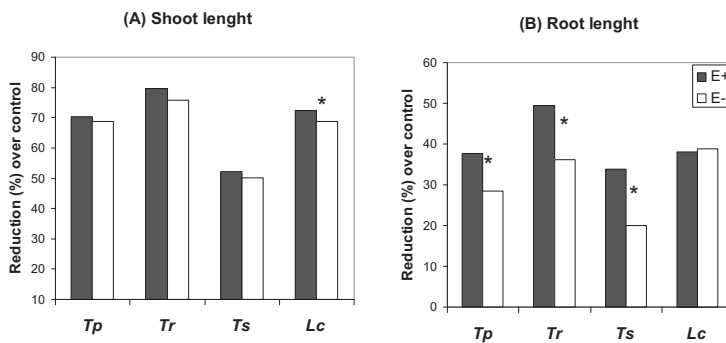


Figure 1. Reduction in shoot (A) and root (B) length (% over control) of four legume species (Tp= *T. pratense*; Tr= *T. repens*; Ts= *T. subterraneum*; Lc= *L. corniculatus*) when growing with *F. rubra* plants infected (E+) and non-infected (E-) by the endophyte *E. festucae*. * = significant differences between E+ and E- pair of means at $P < 0.05$ ($n=20$).

Results and discussion

There were no significant differences between the effect of E+ and E- plants on the emergence of the legume species. The presence of *F. rubra* plants decreased seedling length of the legumes. The inhibitory effect on the shoot length was stronger than on the root length. The inhibition of the shoot length of *L. corniculatus* was significantly ($P < 0.05$) greater when

growing with E+ plants than when growing with E- plants (Fig. 1A). The reduction of the root length of *T. pratense*, *T. repens* and *T. subterraneum* species growing with E+ plants was significantly ($P < 0.05$) greater than when growing with E- plants (Fig. 1B).

Seedling biomass (shoot and root) of the legume species decreased in presence of *F. rubra* plants (Fig. 2). The decrease in shoot biomass was not significantly ($P > 0.05$) affected by the presence of *Epichloë* endophyte in *F. rubra* plants (Fig. 2A). However, the reduction in root length of the four legumes was significantly ($P < 0.05$) greater in presence of E+ than in presence of E- plants (Fig. 2B).

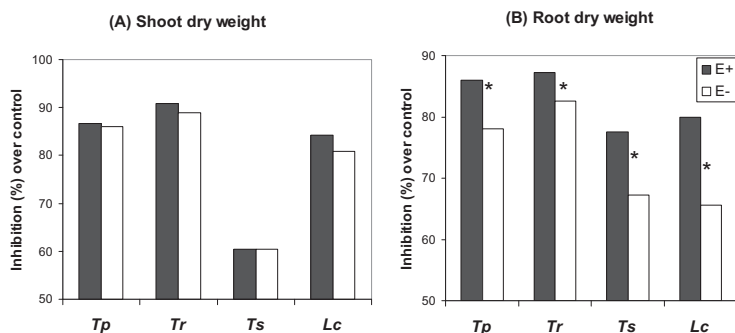


Figure 2. Reduction in shoot (A) and root (B) dry matter (% over control) of four legume species (Tp= *T. pratense*; Tr= *T. repens*; Ts= *T. subterraneum*; Lc= *L. corniculatus*) as affected by the presence of *F. rubra* plants infected (E+) and non-infected (E-) by the endophyte *E. festucae*. * = significant differences between E+ and E- pair of means at $P < 0.05$ (n=20).

Conclusion

When growing with endophyte-infected *F. rubra* plants, root length and root biomass of legumes was lower than when growing with non-infected plants. Therefore, fungal infection by *Epichloë festucae* increases competitiveness of *F. rubra*, mainly by a greater inhibition of root growth of companion legumes.

Acknowledgements

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

References

- Kuldau G. and Bacon C. (2008) Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. *Biological Control* 46, 57-71.
- Fernández-Abascal I., Tárrega R., Luis-Calabuig E. and Marcos E. (2003) Effects of sowing native herbaceous species on the post-fire recovery in a heathland. *Acta Oecologica* 24, 131-138.
- Laser H. and von Boberfeld W.O. (2004) Effect of legume proportion and physiological age on forage quality and the suitability of *Agrostis capillaris* L. and *Festuca rubra* L. for silage making. *Plant, Soil and Environment* 50, 315-323.
- Malinowski D.P., Belesky D.P. and Fedders J.M. (1999) Endophyte infection may affect the competitive ability of tall fescue grown with red clover. *Journal of Agronomy and Crop Science* 183, 91-101.
- Peeters A. (2004) *Wild and Sown Grasses*. FAO and Blackwell, Rome, Italy, 311 pp.
- Zabalgoeazcoa I., Vázquez-de-Aldana B.R., García Criado B. and García Ciudad A. (1999) The infection of *Festuca rubra* by the fungal endophyte *Epichloë festucae* in Mediterranean permanent grasslands. *Grass and Forage Science* 54, 91-95.