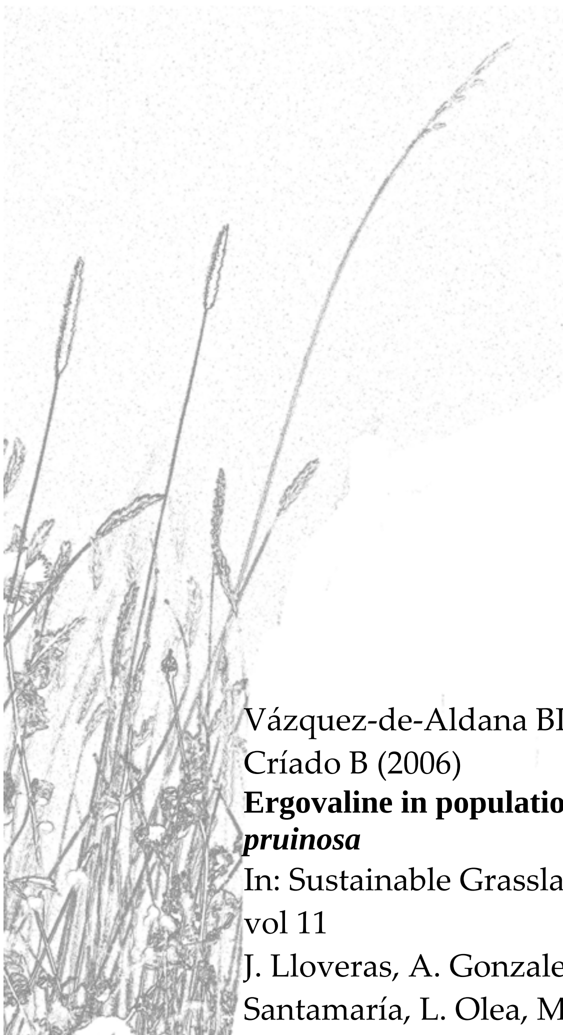

Vázquez-de-Aldana BR, Zabalgogeazcoa I., García-Ciudad A, Garcia-Críado B (2006)

Ergovaline in populations of endophyte infected *Festuca rubra* subsp. *pruinosa*

In: Sustainable Grassland Productivity. Grassland Science in Europe, vol 11
J. Lloveras, A. Gonzalez Rodriguez, O. Vázquez-Yañez, J. Piñeiro, O. Santamaría, L. Olea, M.J. Poblaciones (eds.); European Grassland Federation; pp. 475-477.

3-6 April 2006
Badajoz, Spain

Sustainable Grassland Productivity

Edited by

J. Lloveras
A. González-Rodríguez
O. Vázquez-Yáñez
J. Piñeiro
O. Santamaría
L. Olea
M. J. Poblaciones

EGF

VOLUME 11
GRASSLAND SCIENCE IN EUROPE

Sustainable Grassland Productivity

Proceedings of the 21st General Meeting
of the European Grassland Federation
Badajoz, Spain
3-6 April 2006

Edited by

J. Lloveras
A. González-Rodríguez
O. Vázquez-Yáñez
J. Piñeiro
O. Santamaría
L. Olea
M. J. Poblaciones

Caja de Badajoz

Ergovaline in populations of endophyte infected *Festuca rubra* subsp. *pruinosa*

Vázquez de Aldana B. R., Zabalgogeazcoa I., García Ciudad A. and García Criado B.

Instituto de Recursos Naturales y Agrobiología – CSIC, Apdo 257, 370071 Salamanca, Spain.

Abstract

Festuca rubra subsp. *pruinosa* is a grass whose preferred habitat are coastal cliffs, a harsh environment where plants grow in cavities and are exposed to salt water spray. This grass species is systemically infected by the fungal endophyte *Epichloë festucae* and the interaction is asymptomatic. Endophyte infected grasses produce several alkaloids toxic to herbivores. In a previous work, we showed that an average of 69% of *Festuca rubra* subsp. *pruinosa* plants from cliff ecosystems (in the North Atlantic coast of Galicia region –Spain-) were infected by the endophyte *Epichloë festucae*. The main objective in this study was to determine whether endophyte infected plants of *F. rubra pruinosa* produce the ergovaline alkaloid, which is toxic to large herbivores. For this purpose infected plants of *F. rubra pruinosa*, from four populations and at two different harvests were analysed for the presence of ergovaline. An average of 80% of the analysed plants, which were endophyte infected, contained ergovaline. The alkaloid was detected in the four populations. The concentration ranged from 0.05 – 0.58 μg g^{-1} in the first harvest and between 0.06 and 1.90 μg g^{-1} in the second. The mean ergovaline content increased from the first (0.14 μg g^{-1}) to the second harvest (0.35 μg g^{-1}).

Key words: fungal endophytes, ergovaline, alkaloids, grasses.

Introduction

Festuca rubra subsp. *pruinosa* is a grass whose preferred habitat are coastal cliffs. The environment of these areas is very inhospitable for plant growth mainly due to two characteristics. First, the soil is scarce, and plants grow in cracks and cavities in the rock. Second, the water stress derived from environmental salinity and often from salt-water spray.

Fungal endophytes of the *Epichloë* and *Neotyphodium* genera systemically infect aerial plant tissue of several grass species. Most of these plant-fungus interaction are asymptomatic and the fungus is seed-transmitted. The fungal endophyte *Epichloë festucae* infects several grass species such as *Festuca rubra, F. gigantea, F. glauca*. Endophytic fungi synthesize several biologically active metabolites, notably alkaloids. These alkaloids help in defending the host grass against a range of grass herbivores. The type and amount of alkaloids produced in infected grasses varies with the grass and fungus species involved in the association. For example, *F. rubra* subsp. *rubra* infected by *Epichloë* produces ergovaline, toxic to grazing vertebrates, and peramine, a feeding deterrent to insects (Leuchtmann *et al*., 2000).

Four populations of *F. rubra puinosa* from the northern coast of Galicia, in northern Spain, were analysed for the presence of fungal endopyhtes, and we found that an average of 69% of plants were infected asymptomatically by the endophytic fungi *Epichloë festucae* (Zabalgogeazcoa *et al*., in press). So far, there are no references about the alkaloids produced by this grass–fungus association. The objective of this study was to determine whether the alkaloid ergovaline is produced by infected plants of *F. rubra pruinosa.* For this purpose several plants from different populations and at two different harvests were analysed.

Materials and methods

Festuca rubra subsp. *pruinosa* plants were collected in sea cliff populations in the North Atlantic coast of Galicia (Spain). Four different locations were selected: Cedeira (CED), Torre de Hércules (TDH), Pantín (PAN), and Estaca de Bares (EDV). At each location 15-20 plants were collected leaving a space of at least five meters between plants. Plants were transported to the laboratory (in Salamanca, western Spain) and transplanted into individual pots (in a mixture with peat, sand and perlite). The detection of fungal endophyte infection by *Epichloë festucae* was carried out by microscopic analysis of stem pith scrapings, as well as by isolation of the fungus from plant stems and leaf sheats (Bacon and White, 1994).

In autumn season, infected and non-infected plants were transplanted to an experimental farm, in the province of Salamanca. Plants were irrigated during their establishment but not thereafter, and they were never fertilized. The experimental farm is located in an area characterized by a semiarid continental climate, with low precipitation and high temperatures in spring and summer. Plants were grown several months to obtain enough biomass for chemical analysis of ergovaline. The first sampling was done at the end of May, at heading stage. At this time dry leaves were not present. The second harvest was done one month later, at anthesis stage. At this time, the few dry leaves present were removed. In most plants, the inflorescence was present in a 50% of tillers, but there were plants in an earlier maturity stage where tillers elongated showing the inflorescence were not present. Plant samples were freeze–dried and ground. Ergovaline concentration was determined following the chromatographic method of Hill *et al*., (1993) with the modifications suggested by Yue *et al*. (2000). Ergotamine tartrate (Sigma) was used as internal standard; ergovaline standard was provided by Dr Forrest Smith (School of Pharmacy, Auburn University).

A two-way analysis of variance (ANOVA) was used to determine the effects of plant population and harvest on the ergovaline content of plants.

Results and discussion

The percentage of plants producing ergovaline ranged from 60% in CED population to 100% in EDV population (Table 1). The alkaloid was detected, at both sampling dates, and we did not find plants producing ergovaline only in one harvest. Ergovaline was not detected in non-infected plants.There was a significant effect ($P<0.05$) of harvest on the ergovaline content. The mean concentration of populations increased twice from the first to the second harvest. In CED and PAN populations, the mean in the second harvest was three times the content in the first harvest (Table 1). At both sampling dates, EDV was the population with the lowest ergovaline mean content, and PAN the population with the highest one (Table 1). However, differences between plant populations were not statistically significant ($P>0.05$).The greatest ergovaline concentration (1.9 μg g^{-1}) was detected in PAN population, at the second harvest.

Table 1. Percentage of plants with ergovaline and concentration range (μg g^{-1} dry matter) in *Festuca rubra* subsp *pruinosa* populations infected by *Epichloë festucae*, at two harvests.

Population	Plants with ergovaline (%)	Harvest 1			Harvest 2		
		Mean	Range	SE	Mean	Range	SE
CED	60	0.11	0.06-0.14	0.025	0.32	0.12-0.56	0.130
EDV	100	0.08	0.05-0.15	0.024	0.19	0.09-0.38	0.067
PAN	80	0.21	0.06-0.58	0.124	0.65	0.13-1.9	0.418
TDH	80	0.16	0.07-0.28	0.048	0.24	0.06-0.46	0.093
mean	80	0.14	0.05-0.58	0.055	0.35	0.06-1.9	0.177

The range of concentrations in *F. rubra* subsp. *pruinosa* was higher than that found in *F. rubra* subsp. *rubra* populations from semiarid grasslands (Vázquez de Aldana *et al*., 2004). The trend of increasing ergovaline content with date (maturity stage) is similar to that reported in other infected grass species (Cagas *et al*., 1999). At each harvest, we found that plants without inflorescences (e.g. CED19 = 0.14 μg g^{-1}) can have greater ergovaline content than more mature plants with inflorescences (e.g. TDH4 = 0.07 μg g^{-1}). This suggests that differences in the alkaloid concentration due to plant and fungal genotype could be stronger (more important) than differences due to the maturity stage of the plant.

The ergovaline alkaloid is responsible for fescue toxicosis in livestock, a syndrome mainly characterized by a decrease in weight gain. Most research workers regard 0.40 µg g^{-1} ergovaline in diet as the critical toxic level for cattle (Bony and Delatour, 2001). We found that PAN population in the second harvest had a mean ergovaline content above that level. In the area where plants were collected there are no large grazing herbivores. Therefore, a question arises: which is the function of the ergovaline alkaloid in *F. rubra pruinosa*? In the harsh environmental conditions where plants grow, the cost of harboring the endophyte and the cost of producing this secondary metabolite should bring benefits for these plants, otherwise the percentage of infected plants (an average of 69 %) would be much lower. The alkaloid contents in infected plants can increase under abiotic stress conditions. Thus, it has been reported an increase in ergovaline under water deficit in endophyte infected *Festuca arundinacea* (Belesky *et al*., 1989). This suggests that ergovaline alkaloid may provide an advantage to water-stress and therefore could be related to salinity tolerance.

Acknowledgements

This work was funded by the Spanish Ministry of Education and Science ('PN Investigación Científica Desarrollo e Innovación Tecnológica') + FEDER (AGL2002-02766 AGR-FOR). Thanks are due to Yolanda Arnaiz for technical assistance in the chemical analysis.

References

Bacon C.W. and White J.F. (1994) Stains, media and procedures for analyzing endophytes. In: Bacon C.W., White J.F. (eds) *Biotechnology of Endophytic Fungi of Grasses*. CRC Press, Boca Raton, FL. USA. pp. 47–56.

Belesky D.P., Stringer W.C. and Plattner R.D. (1989) Influence of endophyte and water regime upon tall fescue accessions. II. Pyrrolizidine and ergopeptine alkaloids. *Annals of Botany,* 64, 343-349.

Bony S. and Delatour P. (2001) Relevance and impact of grass endophyte toxins in Europe. In: Paul V.H. and Dapprich P.D. (eds.) The Grassland Conference 2000. *4th International Neotyphodium/grass Interactions Symposium*, Soest, Germany, 207-218.

Cagas B., Flieger M. and Olsovska J. (1999) Concentration of ergot alkaloids in Czech ecotypes of *Lolium perenne* and *Festuca pratensis*. *Grass and Forage Science,* 54, 365-370.

Hill N.S., Rottinghaus G.E., Agee C.S. and Schultz L.M. (1993) Simplified sample preparation for HPLC analysis of ergovaline in tall fescue. *Crop Science,* 33, 331-333.

Leuchtmann A., Schmidt D. and Bush L.P. (2000) Different levels of protective alkaloids in grasses with stroma-forming and seed-transmitted *Epichloë/Neotyphodium* endophytes. *Journal of Chemical Ecology*, 26, 1025-1036.

Vázquez de Aldana B.R., Zabalgogeazcoa I., García Criado B. and García Ciudad A. (2004) Variation of the alkaloids ergovaline and peramine in wild populations of endophyte infected *Festuca rubra*. *Grassland Science in Europe*, 9, 1020-1022.

Yue Q., Johnson-Cicalese J., Gianfagna T.J. and Meyer W.A. (2000) Alkaloid production and chinch bug resistance in endophyte-inoculated chewings and strong creeping red fescues. *Journal of Chemical Ecology,* 26, 279-292.

Zabalgogeazcoa I., Romo M., Keck E., Vázquez de Aldana B.R., García Ciudad A. and García Criado B. The infection of *Festuca rubra* subsp. *pruinosa* by *Epichloë festucae*. *Grass and Forage Science* (in press).