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

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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 pruinosa* from the northern coast of Galicia, in northern Spain, were analysed for the presence of fungal endophytes, and we found that an average of 69% of plants were infected asymptotically 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: Cedreira (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 sheaths (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.

<table>
<thead>
<tr>
<th>Population</th>
<th>Plants with ergovaline (%)</th>
<th>Harvest 1</th>
<th>Harvest 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>SE</td>
</tr>
<tr>
<td>CED</td>
<td>60</td>
<td>0.11</td>
<td>0.06-0.14</td>
</tr>
<tr>
<td>EDV</td>
<td>100</td>
<td>0.08</td>
<td>0.05-0.15</td>
</tr>
<tr>
<td>PAN</td>
<td>80</td>
<td>0.21</td>
<td>0.06-0.58</td>
</tr>
<tr>
<td>TDH</td>
<td>80</td>
<td>0.16</td>
<td>0.07-0.28</td>
</tr>
<tr>
<td>mean</td>
<td>80</td>
<td>0.14</td>
<td>0.05-0.58</td>
</tr>
</tbody>
</table>

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.

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