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**Fungal alkaloids in populations of endophyte-infected *Festuca rubra* subsp. *pruinosa***

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Short title: Fungal alkaloids in endophyte-infected *Festuca rubra*

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## **Abstract**

*Festuca rubra* subsp. *pruinosa* is a grass that grows on coastal cliffs along the Atlantic coast of Europe. Asymptomatic plants of this species are systemically infected by the fungal endophyte *Epichloë festucae*. It is not known whether the alkaloids ergovaline and peramine are produced by the endophyte in *Festuca rubra* subsp. *pruinosa*. Plants from four populations were collected from the northern coast of Galicia (Spain) and examined for the presence of fungal endophytes. Ergovaline and peramine concentrations were analysed over two consecutive years, at two plant growth stages, and in different types of plant tissues. Infected plants of *F. rubra* subsp. *pruinosa* contained ergovaline but do not peramine. Ergovaline was detected in 0.80 of the plants, with concentrations ranging from 0.05 to 1.9  $\mu\text{g g}^{-1}$  dry matter (DM). The differences in ergovaline contents between different types of plant tissue (vegetative and reproductive), plant populations and sampling dates were not statistically significant.

**Keywords:** ergovaline, peramine, grasses, fine fescue, *Epichloë*

## Introduction

*Festuca rubra* L. is a perennial grass, characteristic of soils of moderate nutrient content, which does not compete well with other grasses when high rates of fertilizer are applied. It is somewhat slow-growing, has good drought resistance and is cold- and shade- tolerant. These growth characteristics, together with its fine leaf texture, make *Festuca rubra* one of the most appreciated turf grass species in Europe and North America. Owing to its acceptable nutritive value, it is also appreciated as a forage (Golinski and Kozłowski, 1998). There are several subspecies of *Festuca rubra* adapted to oligotrophic, mesotrophic and saline environments. *Festuca rubra* L. subsp. *pruinosa* (Hack.) Piper is a grass that has adapted to the coastal cliffs of the Atlantic coast of Europe (Auquier, 1971). The environment in these areas is extremely inhospitable to plant growth for two principal reasons. First, there is very little soil and the plants must grow in cracks and cavities in the rock. Second, exposure to salt water spray and wind create conditions similar to water stress. This grass subspecies has a semi-prostrate habit and the surface of its leaves is covered by a waxy coat, presumably to protect the plant from water losses.

Many grasses harbour fungal endophytes belonging to the genera *Epichloë* and *Neotyphodium* (Ascomycota, family Clavicipitaceae). These fungi systemically colonize the intercellular space of leaves and stems. It is common for plants infected by some *Epichloë* species to remain asymptomatic during their life cycle. In this type of infection, the fungus is transmitted vertically to the seeds produced by the infected plants. Other *Epichloë* species cause 'choke disease', in which inflorescence maturation is halted and no seeds are produced (Clay and Schardl, 2002). In populations of *Festuca rubra*, infected by *Epichloë festucae* Leuchtman, Schardl and Siegel, most plants are asymptomatic and produce infected seeds, although a few plants develop choking stromata in all or some of the flowering stems (Leuchtman *et al.*, 1994; Zabalgogezcoa *et al.*, 1999).

Endophyte-infected grasses may contain several kinds of biologically active alkaloids: ergopeptine, lolitrems, pyrrolizidine (loline) and pyrrolopyrazine alkaloids (Porter 1994; Bush *et al.*, 1997). Ergovaline is assumed to be the main causative agent of fescue toxicosis in livestock (Bacon *et al.*, 1977; Schmidt *et al.*, 1982); a syndrome that encompasses symptoms such as loss in live weight, rough hair coat and lower feed intake (Oliver, 2005). However, recent investigations into the ruminal metabolism of the ergot alkaloids have provided results suggesting that ergovaline may not be the only toxin responsible for fescue toxicosis (Hill, 2005). The mycotoxin, lolitrem B, is the major neurotoxin responsible for

staggers syndrome, a seasonal disease of livestock fed *Lolium perenne* L. infected by *Neotyphodium lolii* (Latch, Christensen and Samuels) Glenn, Bacon, and Hanlin (Gallagher *et al.*, 1984). The lolines are mainly active against insects and do not affect mammals (Dahlman *et al.*, 1991). The pyrrolopyrazine alkaloid, peramine, is a feeding deterrent to insects (Rowan, 1993).

The alkaloid profile varies across different endophyte–grass associations depending on both the host grass species and the endophytic fungal species in question. Thus, an endophyte species may produce different types of alkaloid depending on the grass hosting it. For example, *Epichloë festucae* produces ergovaline and lolines in *Festuca gigantea* (L.) Vill. while, in *Festuca glauca* Vill., it produces ergovaline and peramine (Siegel and Bush, 1996). Yue *et al.* (2000) inoculated several fescue species and concluded that the plant genotype apparently plays a more important role in determining the level of synthesis of ergovaline than the genotype of the endophyte. Variations in alkaloid production, associated with the genetic variability of the fungus *Epichloë festucae*, have also been reported (Wilkinson *et al.*, 2000).

Besides the variation in the actual type of alkaloid synthesized, their concentrations are affected by different factors, such as plant and fungus genotypes, plant growth conditions, season, plant part (reviewed by Lane *et al.*, 2000), and environmental conditions such as atmospheric CO<sub>2</sub> concentrations (Hunt *et al.*, 2005). There is also a clear tendency for grass species associated with stroma-forming *Epichloë* species to be free of alkaloids and those that do produce alkaloids to contain only low levels of peramine (Leutschmann *et al.*, 2000). Thus, in *Epichloë festucae*-infected *Festuca rubra*, a host grass where expression of symptoms is variable, choked plants contained no alkaloids whereas in asymptomatic plants ergovaline and peramine were detected (Leutschmann *et al.*, 2000). The results of several studies addressing alkaloid production in asymptomatic associations between *Epichloë* and several grasses of the genera *Agrostis*, *Brachypodium*, *Bromus*, *Elymus*, *Festuca* and *Poa* have shown that peramine is the alkaloid most frequently produced, being detected in 13 of 15 grass species. On the other hand, lolines and lolitrem B are the least frequent alkaloid, detected in only one out of 13 species. Ergovaline was found in 9 out of 15 infected grass species (Siegel *et al.*, 1990; Yue *et al.*, 1997; Leutschmann *et al.*, 2000). The range of variation in the occurrence of alkaloids in the infected host grasses indicates that none of the commonly-examined alkaloids is essential for successful endophyte-grass symbiosis (Lane *et al.*, 2000). Currently no endophyte-infected grass plant producing all four alkaloids is known. *Festuca rubra* subsp. *pruinosa* is naturally and asymptotically infected by the

fungal endophyte *Epichloë festucae* (Zabalgoitia *et al.*, 2006). Furthermore, high rates of infection have been found in plants collected from natural populations on the North Atlantic coast of Galicia, Spain. The objective of this paper is to determine whether ergovaline and peramine alkaloids are produced by endophyte-infected *Festuca rubra* subsp. *pruinosa* plants. For this purpose, plants from four different populations were collected and the alkaloids were analysed at different plant growth stages, in different years and in different plant tissues.

## **Materials and methods**

### **Sample locations**

Plants of *Festuca rubra* subsp. *pruinosa* were collected from four sea-cliff populations located on the North Atlantic coast of Galicia, Spain. These locations were Cedeira (CED), Estaca de Bares (EDB), Pantín (PAN), and Torre de Hércules (TDH). In spring 2001, 15 plants were collected at each location leaving a space of at least 10 metres between plants. The plants were transported to Salamanca, Spain and transplanted first into pots with a mixture of peat, sand and perlite (1:1:1), and later to a field plot on an experimental farm near Salamanca. Plants were watered during their establishment year but not thereafter, and they were never fertilized. The area where the experimental farm is located is characterized by a semi-arid continental climate. Over the last ten years mean annual precipitation has been 430 mm; mean summer precipitation, 63.2 mm; and the temperature ranges between a minimum mean of 0.5 °C in winter and a maximum mean of 28.3 °C in summer.

### **Endophyte detection**

The diagnosis of endophyte infection with *Epichloë festucae* was accomplished by microscopic analysis of stem-pith preparations stained with aniline blue and by isolation of the fungus from plant stems and leaf sheaths (Bacon and White, 1994). Diagnosis was performed at the time of plant collection from the cliffs in spring 2001, and again in 2003.

### **Plant growth conditions and harvesting**

After several months' growth in the field, a total of 20 infected plants (five from each population) and 8 naturally endophyte-free plants (two from each population) were randomly selected for alkaloid analysis. In 2002, plants were harvested twice: the first harvest was conducted at the end of May, and the second one at the beginning of July. In

2003, plants were harvested only at the end of May. At each harvest one half of the plant was cut, and soil debris and dead tissue removed.

Samples of different plant parts were obtained from the harvested plants as follows. All plant samples collected in July 2002 were separated into reproductive parts (culms) and vegetative parts (leaves and pseudostems). The number of reproductive tillers varied among plants, such that some plants did not have enough reproductive tillers for chemical analysis. In these cases, only vegetative samples were used. In the harvest of May 2002, one plant randomly selected from each population was separated into leaf blades and leaf sheaths (or pseudostem). The remaining samples were analysed without this type of separation.

### **Alkaloid analysis**

Each freeze-dried and ground sample was analysed for ergovaline and peramine alkaloids. The ergovaline concentration was determined using a modification of the methods described by Hill *et al.* (1993) and Yue *et al.* (2000). A 1.0 g sample was extracted in 20 ml of  $\text{CHCl}_3$  and 1 ml of 0.5 mM NaOH for two hours. One hundred microlitres of an internal standard solution ( $10 \mu\text{g ml}^{-1}$ ) of ergotamine ditartrate (Sigma Chemical Co, St Louis, USA) were added to the sample prior to extraction. The mixture was vacuum-filtered through Whatman n° 2 filter paper. An aliquot of 10 ml of filtrate was passed through a 500 mg Ergosil (Analtech, Newark, DE, USA) solid-phase column preconditioned with  $\text{CHCl}_3$ . Plant pigments were removed with 5 ml of  $\text{CHCl}_3$ : acetone (1:3). The sample was eluted with 2 ml of methanol and vacuum-concentrated, redissolved in 1 ml of methanol and filtered through a  $0.22 \mu\text{m}$  nylon filter. Extracts were chromatographed with a Waters 2690 system (Waters Corporation, Barcelona, Spain) with a Xterra MS C18 Waters column (4.6 x 100 mm) and a guard column (3.9 x 20 mm) of the same characteristics. The initial solvent gradient was 35% acetonitrile in 0.01 M ammonium acetate buffer (pH=7.6) with a flow rate of  $0.8 \text{ ml min}^{-1}$ . The gradient was adjusted through time programming as follows: step 1, 35% to 50% acetonitrile in a 20 min linear gradient; step 2, 50% for 5 min; step 3, 50% to 90% in a 5 min linear gradient; step 4, 90% to 35% in a 5 min linear gradient. Ergovaline was detected by fluorescence spectrophotometry with an excitation wavelength of 250 nm and an emission wavelength of 420 nm (Waters Fluorescent Detector 2475; Waters Corporation, Barcelona, Spain). A standard was prepared by adding ergovaline (from Forrest Smith, Auburn University, USA) to a 1.0 g sample of a non-infected *Festuca rubra* plant free of ergovaline, which was treated as described above. The limit of detection was  $0.01 \mu\text{g g}^{-1}$ . Peramine was

determined using the HPLC method described by Barker *et al.* (1993) and Yue *et al.* (2000). A freeze-dried and ground sample (100 mg) was extracted in 3 ml of 30% isopropanol for 30 min at 90°C. The mixture was centrifuged and the extract was passed through a preconditioned Varian Bond Elut carboxylic acid (CBA) column packed with 100 mg of adsorbent. After a wash of the column with 1-2 ml of methanol, peramine was eluted with 1 ml of 5% formic acid in 80% aqueous methanol. The extract was filtered through a 0.22 µm nylon filter and chromatographed in a Waters 2690 system with a Nova Pak C18 Waters column (3.9 x 150 mm). The isocratic mobile phase consisted of 18% (v:v) acetonitrile in a guanidine carbonate (10 mM)-formic acid buffer. Detection was performed with a Photodiode Array Detector (PDA) Waters 2996 set at 280 nm. Peramine standard was a gift from G. Lane (AgResearch, New Zealand). The limit of detection was 0.8 µg g<sup>-1</sup>.

When samples were separated into plant parts, the alkaloid content in the whole plant was numerically calculated on the basis of the concentration and dry matter (DM) content of each part.

### **Statistical analysis**

A two-way ANOVA assay was used to analyse the effect of plant population and sampling date (May 2002, July 2002 and May 2003). The effect of plant tissue type (vegetative or reproductive) was analysed using data from July 2002 by means of a two-way ANOVA, with tissue type and plant population as factors. The effect of leaf part (blade or sheath) was analysed in four selected samples from May 2002 by means of a t-test for independent samples. SPSS 12.0 was used to perform all statistical analyses.

## **Results**

### **Infection frequencies**

In the four populations analysed, infection rates ranged from a proportion of 0.56 in the EDB population to 0.79 in the CED population; the average was 0.69 (Table 1). The endophytic fungus had previously been identified as *Epichloë festucae* (Zabalgoitia *et al.*, 2006).

### **Ergovaline concentrations**

Ergovaline was detected in 16 out of 20 infected plants analysed. All plants producing ergovaline did so at each of the three harvests, except for one plant from the TDH population in which ergovaline was not detected in 2003. Ergovaline was not detected in non-infected plants. In the four populations, the proportion of plants containing ergovaline ranged from

0.60 in CED to 1.0 in EDV. The highest concentration was detected in the PAN population (Table 1). In all four populations, plants with low concentrations of ergovaline ( $0.05 \mu\text{g g}^{-1}$  DM) were found. The mean ergovaline concentration ranged from  $0.08 \mu\text{g g}^{-1}$  to  $0.65 \mu\text{g g}^{-1}$  DM. For whole populations, the mean ergovaline concentration ranged from  $0.14 \mu\text{g g}^{-1}$  DM at the May 2002 harvest to  $0.35 \mu\text{g g}^{-1}$  DM at the July 2002 harvest. A two-way analysis of variance did not reveal any significant differences ( $P>0.05$ ) in the ergovaline concentrations among plant populations, sampling dates or their interaction.

Table 2 shows the ergovaline concentration in vegetative and reproductive tillers of *F. rubra* subsp. *pruinosa* plants harvested in July 2002. The concentration ranged from  $0.05$  to  $0.50 \mu\text{g g}^{-1}$  DM in vegetative tillers (mean  $0.20 \mu\text{g g}^{-1}$  DM) and from  $0.06$  to  $0.56 \mu\text{g g}^{-1}$  DM in reproductive tillers (mean  $0.25 \mu\text{g g}^{-1}$  DM). Two-way analysis of variance revealed no significant effect ( $P>0.05$ ) of plant tissue type, population or their interaction on the ergovaline concentration. The mean ergovaline concentration (s.e. of mean in parentheses) of the four selected samples was  $0.10$  ( $0.010$ )  $\mu\text{g g}^{-1}$  DM in leaf blades, and  $0.38$  ( $0.117$ )  $\mu\text{g g}^{-1}$  DM in leaf sheaths. A t-test revealed that differences in ergovaline concentrations between these leaf parts were statistically significant ( $P<0.05$ ).

### **Peramine concentrations**

The alkaloid, peramine, was not detected in any sample of *F. rubra* subsp. *pruinosa* infected by *Epichloë festucae*.

### **Discussion**

The results of this study show that plants of *Festuca rubra* subsp. *pruinosa* infected by *Epichloë festucae* contain ergovaline but not peramine. Peramine was not detected in any of the 78 plant samples from the four different populations on any harvest date or in any type of tissue. The variation in the sample set of *F. rubra* subsp. *pruinosa* plants was large enough to detect peramine alkaloid, if present. Some studies have analysed a low number of samples of infected plants (less than 10 plants) with variation in growth status (Leuchtman *et al.*, 2000) but not such a range in populations as in this study. This variation is important since a certain proportion of plants may not produce ergovaline. Ergovaline was detected in more than half of the samples of each population and on different harvest dates. In addition, almost all plants producing ergovaline did so at all harvests.

The alkaloid profile in the grass–endophyte associations depends on both the grass and fungal species. Ergovaline and peramine are the most frequent alkaloids in *Festuca* hosts of *Epichloë festucae* (Siegel *et al.*, 1990; Yue *et al.*, 1997; Leuchtman *et al.*, 2000; Vázquez de Aldana *et al.*, 2004). As shown in Table 3, which also includes the results of the present study, of the 10 plant host–fungus associations considered, peramine has been detected in 8 (proportionately 0.80) associations and ergovaline in 9 proportionately (0.90) associations. Lolitrem B was detected only in *Festuca longifolia* Thuill. and lolines only in *Festuca gigantean* (L.) Vill. Within the subspecies of *Festuca rubra*, only the occurrence of ergovaline and peramine alkaloids has been reported. Both alkaloids have been detected in seven of 10 *Festuca* species infected by *Epichloë festucae*. In *Festuca rubra* L. subsp. *rubra* (Gaud.) Hayek both alkaloids were detected by Yue *et al.* (1997) and Vázquez de Aldana *et al.* (2004). However Siegel *et al.* (1990) detected ergovaline but not peramine. In this case, the authors did not indicate the number of plants analysed, although the number was probably low since they analysed several different species.

A case, in which a *Festuca-Epichloë festucae* association contained only one alkaloid, has been reported. *Festuca rubra* L. subsp. *litoralis* (Mey.) Auquier, artificially infected by an isolate obtained from an unknown cultivar of *Festuca rubra* L. subsp. *conmutata* Gaud. produced only ergovaline (Siegel *et al.*, 1990). *Festuca rubra* subsp. *litoralis* also grows in coastal ecosystems. Similarly, in the present study it was found that *F. rubra* subsp. *pruinosa* contained ergovaline but not peramine.

In this study the alkaloid concentrations in plant samples, harvested directly from their original location on sea cliffs, were not analysed because the plant biomass collected was not large enough for chemical analysis. Several environmental factors can influence alkaloid concentrations in plants and it could be speculated that the effects of this may be underestimated if the plant samples are not analysed at the site of collection. It has been reported that ergovaline concentration may increase when infected plants are subject to stress conditions such as drought (Belesky *et al.*, 1989; Lane *et al.*, 1997b). Also, application of N or P fertilizer may lead to increases in ergovaline concentrations in infected *Festuca arundinacea* Schreb. and *Lolium perenne* (Arechavaleta *et al.*, 1992; Lane *et al.*, 1997b; Malinowski *et al.*, 1998). On the other hand, peramine concentrations did not differ among soil moisture and nutrient treatments (Faeth *et al.*, 2002). In this study, plants were grown in field plots without irrigation or fertilizer in a semi-arid environment and in a soil with low organic matter (1.24%) and N (0.067%) contents. These conditions are different from those

found on the sea cliffs where plants were collected. However, in both stress conditions were present.

The ergovaline concentration detected in *F. rubra* subsp. *pruinosa* plants (range 0.05-1.9  $\mu\text{g g}^{-1}$  DM; mean 0.23  $\mu\text{g g}^{-1}$ ) was much higher than the levels found in *Festuca rubra* subsp. *rubra* plants from semi-arid grasslands of Western Spain (range 0.05-0.25  $\mu\text{g g}^{-1}$  DM; mean 0.13  $\mu\text{g g}^{-1}$  DM) grown under the same environmental conditions on field plots next to those of *F. rubra* subsp. *pruinosa* used for this study (Vázquez de Aldana *et al.*, 2004). The ergovaline concentrations are within the levels reported for several *Festuca rubra* subspecies in different growth conditions (Siegel *et al.*, 1990; Yue *et al.*, 1997; Leuchtman *et al.*, 2000) (Table 3). Analysis of variance did not reveal any significant difference in the ergovaline concentration of *F. rubra* subsp. *pruinosa* among plant populations. This means that the variability among individual plants within a plant population is greater than the variability between plant populations. Diversity in quantitative alkaloid accumulation is expected to be greater in natural ecosystems than in agricultural or cultivated systems, mainly due to increased genetic heterogeneity of both host grass and endophyte.

There was no significant difference in the ergovaline concentration with sampling date. It has been reported that the ergovaline concentration in *Festuca arundinacea* plants infected by *Neotyphodium coenophialum* (Morgan-Jones & Gams) Glenn, Bacon and Hanlin increase when seed-heads emerge (Belesky *et al.*, 1988; Agee and Hill, 1994). The absence of differences in this study between the two sampling dates at different growth stages suggests that the differences in the maturity of the plants were not sufficiently large to detect differences in ergovaline concentrations. Neither was there a significant difference in ergovaline concentration between reproductive and vegetative tillers. These results again suggest considerable variability in ergovaline concentrations among individual plants. In the above-cited works, and indeed in most other studies, only one or two cultivars of a given species were used (mainly *F. arundinacea* and *L. perenne*) and hence this variability was not included. However, higher ergovaline concentrations were found in leaf sheaths than in blades. This accumulation of ergovaline in leaf sheaths has been observed previously in *F. arundinacea* infected by *N. coenophialum* (Rottinghaus *et al.*, 1991) and in *L. perenne* infected by *N. lolii* (Lane *et al.*, 1997a).

A concentration of 0.40  $\mu\text{g g}^{-1}$  DM of ergovaline in the diet is considered to be the critical level above which clinical symptoms of fescue toxicosis may be observed (Bony and Delatour, 2001). Several plants in this study had ergovaline concentrations above this critical value. Furthermore, in the second harvest the mean ergovaline concentration of the PAN

population was above the critical level. In the area where the plants were collected, there are no large grazing herbivores. And, hence, the following question arises: what role does ergovaline play in *Festuca rubra* subsp. *pruinosa* plants? The effects of fungal alkaloids are well known in two agronomic grasses, *F. arundinacea* and *L. perenne*, where they are directly linked to livestock toxicoses (Bacon *et al.*, 1977) and neurological disorders in sheep, cattle, and horses (Fletcher and Harvey, 1981). Ergopeptine alkaloids are primarily active against vertebrate herbivores although some insecticidal activity has also been reported (Siegel and Bush, 1996). Several studies (Clay and Schardl, 2002; Popay and Bonos, 2005) have addressed the alkaloid-induced resistance to herbivores, not only domestic herbivores but also small mammals, insects and nematodes, of a wide range of both cultivated and wild endophyte-infected grasses. This defence against herbivores may provide a selective advantage to infected vs. non-infected plants. It is likely that ergovaline production in *F.* subsp. *pruinosa* plants infected by *Epichloë festucae* would presumably play a role in plant defence against wild herbivores.

Additionally, an increase in ergovaline concentration in endophyte-infected *Festuca arundinacea*, challenged by a water deficit, has been reported (Belesky *et al.*, 1989; Arechavaleta *et al.*, 1992). This suggests that ergovaline may provide an advantage in water-stress conditions and could, therefore, be related to salinity tolerance. Several studies have shown that endophyte-infected grasses are more tolerant of drought and other abiotic stresses (Malinowski and Belesky, 2000). In an experiment designed to test whether the presence of the fungus affected the response of *F. rubra* subsp. *pruinosa* plants to salinity, it was found that endophyte infection did not influence the biomass production of plants in conditions of salinity (García Ciudad *et al.*, 2002; Zabalgogezcoa *et al.*, 2006). In the harsh environmental conditions where the plants were found, the cost of harbouring the endophyte and the cost of producing this secondary metabolite should bring benefits for them; otherwise, the proportion of infected plants and the proportion of plants producing ergovaline would be much lower.

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**Table 1** Proportion of infected plants and ergovaline content ( $\mu\text{g g}^{-1}$  DM) in four populations in northern Spain (CED, EDB, PAN and TDH) of *Festuca rubra* subsp. *pruinosa* infected by *Epichloë festucae* at three sampling dates.

Plant Population	Proportion of Infected plants	Sampling date	Plants with ergovaline/plants analyzed	Range ( $\mu\text{g g}^{-1}$ DM)	Mean ( $\mu\text{g g}^{-1}$ DM)	s.e. of mean
CED	0.789	May 2002	3/5	0.06 - 0.14	0.11	0.025
		July 2002	3/5	0.12 - 0.56	0.32	0.130
		May 2003	3/5	0.05 - 0.24	0.15	0.055
EDB	0.562	May 2002	5/5	0.05 - 0.15	0.08	0.023
		July 2002	5/5	0.09 - 0.38	0.19	0.067
		May 2003	5/5	0.05 - 0.27	0.14	0.052
PAN	0.774	May 2002	4/5	0.06 - 0.58	0.21	0.124
		July 2002	4/5	0.13 - 1.90	0.65	0.417
		May 2003	4/5	0.05 - 0.70	0.27	0.146
TDH	0.625	May 2002	4/5	0.07 - 0.28	0.16	0.048
		July 2002	4/5	0.06 - 0.46	0.24	0.093
		May 2003	3/5	0.05 - 0.44	0.22	0.122
Mean	0.687	May 2002		0.05 - 0.58	0.14	0.035
		July 2003		0.06 - 1.90	0.35	0.117
		May 2002		0.05 - 0.70	0.20	0.049
Overall			16/20	0.05 - 1.90	0.23	0.046

**Table 2** Ergovaline concentration ( $\mu\text{g g}^{-1}$  DM) in vegetative and reproductive tillers of *Festuca rubra* subsp. *pruinosa* populations (CED, EDB, PAN and TDH) harvested in July 2002.

Plant Population	Vegetative tillers			Reproductive tillers		
	Range	Mean	s.e. of mean	Range	Mean	s.e. of mean
CED	0.09 - 0.30	0.19	0.105	0.12 - 0.28	0.20	0.078
EDB	0.10 - 0.25	0.18	0.043	0.06 - 0.53	0.26	0.141
PAN	0.12 - 0.25	0.19	0.065	0.17 - 0.56	0.37	0.195
TDH	0.05 - 0.50	0.22	0.089	0.09 - 0.43	0.22	0.076
Mean	0.05 - 0.50	0.20	0.039	0.06 - 0.56	0.25	0.053

**Table 3** Alkaloid types occurring in different grasses of the genus *Festuca*asymptomatically infected by *Epichloë festucae* endophytes.

Host grass	Origin of endophyte	Alkaloid type ( $\mu\text{g g}^{-1}$ DM)				Reference
		lolines	peramine	lolitrem B	ergovaline	
<i>F. gigantea</i>	NI	36 - 224	2.8 - 5.2	ND	–	Leuchtmann <i>et al.</i> (2000)
<i>F. glauca</i>	NI	–	5	–	0.8	Siegel <i>et al.</i> (1990)
<i>F. longifolia</i>	NI	–	22	4	0.9	Siegel <i>et al.</i> (1990)
<i>F. ovina duriuscula</i>	NI	ND	7.8 - 37.6	ND	2.57 - 3.50	Yue <i>et al.</i> (1997)
<i>F. ovina ovina</i>	NI	ND	0.0 - 1.1	ND	0.61 - 2.28	Yue <i>et al.</i> (1997)
<i>F. rubra</i>	NI	–	0 - 15.8		0 - 1.4	Leuchtmann <i>et al.</i> (2000)
<i>F. rubra conmutata</i>						
Longfellow	NI	–	18	–	0.5	Siegel <i>et al.</i> (1990)
Enjoy	NI	–	16	–	–	Siegel <i>et al.</i> (1990)
Jamestown II	NI	ND	5.3 - 32.9	ND	1.68 - 5.42	Yue <i>et al.</i> (1997)
<i>F. rubra litoralis</i>	AI	–	–	–	0.8	Siegel <i>et al.</i> (1990)
<i>F. rubra rubra</i>	NI	–	–	–	1.1 - 1.3	Siegel <i>et al.</i> (1990)
	NI	ND	0.0 - 2.5	ND	0.33 - 3.26	Yue <i>et al.</i> (1997)
		ND	0.0 - 8.2	ND	0.0 - 0.25	Vazquez de Aldana <i>et al.</i> (2004)
<i>F. rubra pruinosa</i>	NI	ND	–	ND	0.0 - 1.9	This paper

NI, naturally infected

AI, artificially infected by a *F. rubra conmutata* isolate obtained from an unknown cultivar

–, not detected

ND, not determined