

Incidence of *Neotyphodium* endophytes in Spanish perennial ryegrass accessions

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Abstract

Fifty-four accessions of perennial ryegrass collected in North Spain were examined for the presence of *Neotyphodium*. One hundred seeds per accession were examined. Seventy-two per cent of the North Spanish accessions examined harbored endophyte, showing the widespread incidence of infection. Infection rates were generally lower than 50% and rarely extreme. Infection levels above 50% were mainly present in accessions collected in the warmest and driest locations of our study. Relationships between endophyte infection and climatic characteristics were studied using Spearman's correlations. Significant correlations were found between endophyte infection, the water supply deficit ($r = 0.43$) at $P < 0.01$ and

with the average absolute maximal temperature ($r = 0.34$) at $P < 0.05$.

Key words: *Neotyphodium* endophytes, climatic characteristics, endophyte infection, genetic resources, *Lolium perenne*.

Introduction

Perennial ryegrass is an important species of forage grass and can harbour fungal endophytes, some of them belonging to *Neotyphodium* (formerly *Acremonium*). The endophytes of perennial ryegrass and tall fescue are important in New Zealand and the United States, respectively, where they have been studied for many years. It is now well established that they are mutualistic (Clay 1986) and that they improve resistances to abiotic and biotic stress in grasses (Van Heeswijck and McDonald, 1992).

A survey of ryegrass endophyte was conducted by Lewis and Clements (1986). They found 14 out of 61 endophyte-infected samples of a sward collected at 52 widespread sites in the United Kingdom. Latch et al. (1987) studied the incidence of endophytes in European wild accessions and commercial cultivars of *Lolium* and

Festuca. These authors reported that wild accessions of *Lolium perenne* often harbor endophyte whereas infection is rare in cultivars of this species. At least 72 per cent of French perennial ryegrass accessions harbor endophyte and endophyte infection levels within accessions are rarely extreme (Ravel et al. 1994). Regional variation in endophyte infection levels have been reported by White et al. (1993) for many grasses, and by Ravel et al. (1994) for perennial ryegrass. There is no information available about endophyte infection on wild accessions of *Lolium perenne* in Spain. The aims of this work were to assess the geographic distribution and level of infection of *Neotyphodium* endophytes in 54 wild accessions of perennial ryegrass in North Spain and to relate endophyte infection with some climatic characteristics.

Materials and Methods

A sample of 54 accessions of perennial ryegrass collected as seeds in North Spain (provinces of: A Coruña, Lugo, Ourense, Pontevedra, Asturias, León, Cantabria, Vizcaya and Guipuzcoa) were examined for

endophyte presence. The collection and evaluation designs have been fully described by Oliveira and Charmet (1988) and by Arbones and Oliveira (1995).

Recalling the main steps could be useful, however. Samples were collected in the summers of 1985, 1991 and 1996. Collectors prospected the Galicia administrative region in the summer of 1985 and Northern Spain in the summers of 1991 and 1996. Collectors sampled wild ryegrass as regularly as possible to obtain an extensive sample of the possible variation of the natural species. Geographic coordinates of the collection sites are available on request. At each collecting site, seeds from at least 50 plants were taken from an ecologically homogeneous area of 10-1000 m². These conditions are supposed to yield a sample of seeds representative of the original panmictic accession (Tyler 1987). Information about collection sites was taken at the time of collection: general habitat, grassland management, latitude, longitude and altitude. Seeds of all plants were bulked, without balancing for the contribution of each plant. Seed was stored in manila packets at 4°C and 45-50% relative humidity. Climatic data were obtained from the closest meteorological recording stations to

the individual seed sampling sites (MAPA 1986). Climatic data were: 25-year average annual temperature (T), 25-year average absolute minimal temperature (TMIN), 25-year average absolute maximal temperature (TMAX), 25-year annual rainfall (P), evapotranspiration (cumulated data from March to October, noted ETP) and water supply deficits (WDEF).

One hundred seeds from each sample were soaked in 50 g l⁻¹ NaOH for 16 h, rinsed with tap water, dehulled, stained with 5 g l⁻¹ aniline blue, and then squashed with a coverslip. Each slide was examined under a microscope with 200x magnification. The number of seeds containing a Neotyphodium-like endophyte (Hinton and Bacon, 1985) was recorded as infection percentage of the sample. Examination of 100 seeds led to estimations of endophyte infection percentages with confidence intervals < 20%. These estimates could not be improved by examination of more seeds due to the only small quantity available.

After determining that the endophyte infection and the six climatic characteristics described above were markedly non normal, Spearman`s correlations were calculated between endophyte infection (EI) and every climatic characteristic.

Results and Discussion

Of the 54 perennial ryegrass accessions examined, 39 (72%) accessions were infected with endophyte at a mean infection frequency of 33.3% (s.e. = 3.9). The true infection frequency varied with 95% of confidence between 25.5 and 41.1%. This level of endophyte infection (72%) is similar to the level of 64% reported by Ravel et al. (1994) in French wild accessions of perennial ryegrass.

The infection level varied from 0% to 91%. Most infected accessions showed a low infection value: 42 accessions had an infection rate below 50% whereas 12 accessions had an infection rate above 50% (Figure 1). These results showed that most North Spanish wild accessions harbored endophyte, although mostly at low to intermediate levels (zero to 50%). Infection levels above 50% were mainly present in accessions collected in the warmest and driest locations of our study (Figure 2).

The Spearman's correlations between endophyte infection (EI) and climatic characteristics (Table 1) revealed two climatic variables that were

significantly related to EI: WDEF ($r = 0.43$) and TMAX ($r = 0.34$). The former correlation was significant at $P < 0.01$ and the latter at $P < 0.05$. Correlations between endophyte infection and the other climatic characteristics were not significant. Work in the United States has shown that endophyte infection improves drought tolerance of tall fescue (West 1994). The mechanisms proposed to explain drought tolerance within the symbiosis are diverse. Early work suggests that root growth of tall fescue is greater in symbiotic plants (West et al. 1989). Symbiotic tall fescue has also been found to express enhanced osmotic adjustment and turgor maintenance under water stress compared with non-symbiotic fescue (Elmi et al. 1992). The high frequency of infected plants of perennial ryegrass in areas of high water deficit obtained in this study may confer a competitive advantage of infected plants in some areas of Northern Spain where summer drought occurs regularly and is a severe restriction on the growth of perennial ryegrass. Further studies are necessary to determine whether high endophyte infection of perennial ryegrass in areas of high water deficit is related to drought survival.

As far as we are aware, this is the first report of endophyte infection on *Lolium perenne* wild accessions in Spain.

Acknowledgments

We appreciate the technical assistance of J. Collar from the Laboratorio Agrario y Fitopatológico.

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Table 1: Spearman's correlations between endophyte infection (EI) and climatic variables: average annual temperature (T), average absolute minimal temperature (TMIN), average absolute maximal temperature (TMAX), annual rainfall (P), evapotranspiration (ETP) and water supply deficits (WDEF).

Variable	EI
EI	1.00
T	-0.01
TMIN	-0.26
TMAX	0.34*
P	-0.22
ETP	0.07
WDEF	0.43**

Note: N = 39. **P < 0.01, *P < 0.05

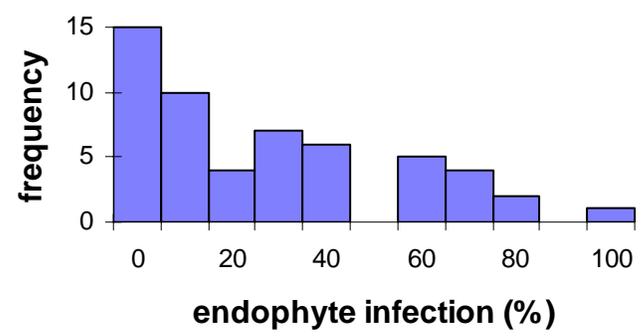


Figure 1: Histogram of endophyte infection percentages in 54 Spanish wild accessions of perennial ryegrass.

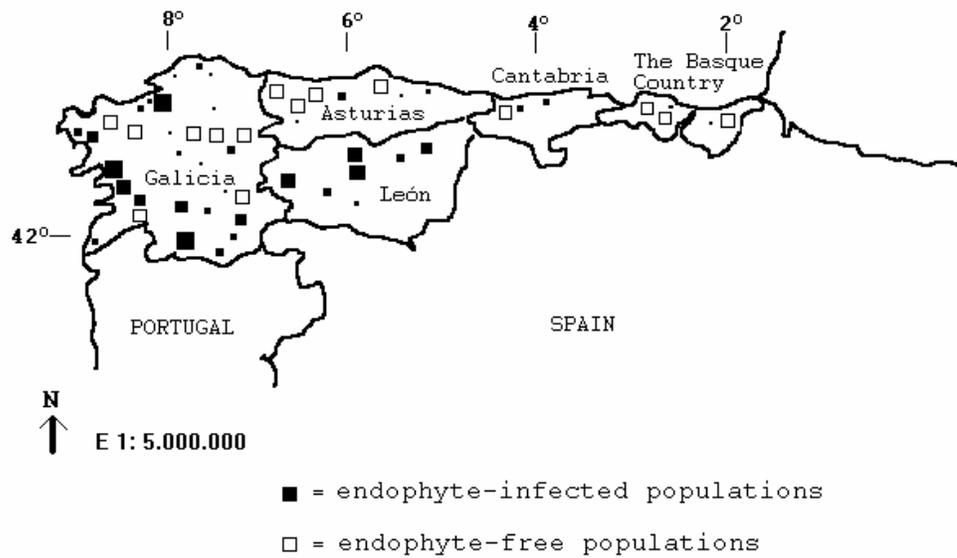


Figure 2: Endophyte infection levels in 54 Spanish wild accessions of perennial ryegrass: symbol size is proportional to the infection levels.