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A survey of grass species infected by fungal endophytes in dehesa pastures

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SUMMARY - *Epichloë* and *Neotyphodium* fungal endophytes infect several grass species. Endophytic infection is correlated with greater drought and invertebrate pest resistance in host plants, and with intoxications in livestock due to the production of alkaloids by the fungi. To find out which grass species in dehesa systems are infected by fungal endophytes, a survey was made in the province of Salamanca (Spain). Plant samples were microscopically examined for the presence of intercellular mycelium, and fungal endophytes were isolated and identified. Several grass genera were found to be infected by endophytic fungi. High rates of infection were observed in several species of *Festuca*. Other genera which hosted endophytes were *Lolium*, *Holcus*, and *Alopecurus*. In spite of being advantageous for the plants, the antiherbivore alkaloids present in infected grasses may be an antinutritional factor to consider in dehesa pastures.

Key words: Grasses, dehesas, *Neotyphodium*, *Epichloë*, endophytes.

RESUME *- "Etude des espèces de graminées contaminées par des champignons endophytes dans des pâturages type Dehesa". Plusieurs espèces de graminées sont contaminées par des champignons endophytes* Epichloë *et* Neotyphodium. *Chez les plantes hôtes, la contamination endophyte est corrélée avec une plus grande résistance à la sécheresse ainsi qu'aux invasions d'invertébrées et avec les intoxications chez le bétail dues à la production d'alcaloïdes par le champignon. Une étude a été menée pour identifier les espèces de graminées contaminées par des champignons endophytes dans les systèmes de "dehesa" (pâturage) de la province de Salamanca (Espagne). Les échantillons des plantes ont été examinés au microscope pour observer la présence de mycélium intercellulaire de champignons endophytes. Les champignons ont été isolés et par la suite identifiés. Plusieurs genres de graminées sont contaminés par des champignons endophytes. Le plus fort degré de contamination est observé chez les espèces de* Festuca. *Les genres* Lolium, Holcus *et* Alopecurus *hébergent aussi des champignons endophytes. Il est intéressant de considérer la présence d'alcaloïdes chez les espèces de graminées contaminées dans les pâturages de dehesa car ce facteur antinutritionnel pour les herbivores suppose des avantages pour les plantes.*

Mots-clés *: Graminées, dehesas,* Neotyphodium, Epichloë, *endophytes.*

Introduction

Clavicipitaceous fungal endophytes of the genera *Neotyphodium* and *Epichloë* systemically infect the intercellular spaces of plants belonging to several grass genera. During the vegetative life cycle of the plant, these fungi do not induce any symptom, however, when the plant reproductive cycle starts, grass endophyte interactions can be of three types, depending on the species involved in the host-fungus association. In type I interactions, during the plant reproductive cycle an external fungal stroma prevents the emergence of the inflorescence, thus sterilizing the plant. This symptom is known as "choke disease" of grasses. Type II interactions are characterized because a few infected plants in a population are sterilized by stromata, but most infected plants remain asymptomatic, and produce seeds which are infected by the fungus. When those infected seeds germinate, they give rise to infected plants. In type III interactions, infected plants do not show any symptom, and the fungus is seed transmitted to the next generation (White, 1988).

In these interactions in which infected plants remain asymptomatic and the fungus is seed transmitted (type II and III), endophyte infection may be beneficial for the plant host. It has been reported that *Festuca* and *Lolium* species infected by *Neotyphodium* and *Epichloë* endophytes are more resistant to several species of insects than uninfected plants. Also, seed production and germination is greater in

infected *F. arundinacea* and *L. perenne* plants, and infected *F. arundinacea* plants are more resistant to drought stress than uninfected plants (Siegel *et al.*, 1987; Clay, 1987).

The interest in grass-endophyte interactions started when it was demonstrated that a series of health problems occurring in grazing livestock, known as fescue toxicosis, were caused by the consumption of *F. arundinacea* infected by the endophyte *N. coenophialum* (Bacon *et al.*, 1977). Similar problems also occur in sheep fed with *Lolium perenne* infected by *N. lolii*. These problems are caused by the production of toxic alkaloids by *Neotyphodium* and *Epichloë* endophytes in infected plants. Endophyte-related livestock toxicoses are considered a serious problem in New Zealand and the United States, where ryegrass and tall fescue pastures are the main component of the livestock diet.

The objective of this study was to find out if grass species present in dehesa grasslands are infected by fungal endophytes. The presence of such fungi in grass species could contribute negatively to the nutritional quality of these pastures.

Materials and methods

From May 1996 until June 1997, grass plants were collected during the growth season. The plants were obtained in 18 dehesa farms of the province of Salamanca, in western Spain. The presence of fungal endophytes was determined by microscopical examination of stem pith scrapings stained with aniline blue (Bacon *et al.*,1977). In species in which endophytic mycelium was detected by microscopic examination, the fungi were isolated from a limited number of samples in order to obtain pure cultures using the method described by Bacon *et al.* (1977). Morphological characters of the pure cultures were used for the taxonomic identification of the fungal endophytes (White and Morgan-Jones, 1987).

Results and discussion

Grass species belonging to 23 genera were sampled and analyzed for the presence of fungal endophytes (Table 1). Except when plants showing choke disease were found, all other plants collected were asymptomatic and bore flowering panicles. Endophytic infections were detected in five genera: *Festuca, Lolium, Holcus, Dactylis and Alopecurus.*

The most significant rates of endophytic infection were observed in the genus *Festuca*. In all 10 locations where *F. arundinacea* plants were found, infected plants occurred. In this species 67% of the analyzed plants had endophytes associated. The endophyte of *F. Arundinacea* was isolated from 8 different plants and identified in pure culture as *N. coenophialum.* The *F. arundinacea-N. coenophialum* association is of type III, and all infected plants were asymptomatic. Infected *F. rubra* plants were found in all 18 locations sampled in this study, and 71% of the plants of this species were endophyte-infected. Pure cultures of the fungi isolated from 24 plant samples were identified as *Epichloë festucae*. This plant-fungus interaction is of type II, and of all infected plants only 11 showed stromata which prevented flowering, in these plants not all flowering culms showed choke symptoms, some were asymptomatic.

Asymptomatic *Lolium perenne* plants infected by *N. lolii* were also found (Table 1). We think that some *Lolium* plants found in dehesas may have its origin in seed from hay given to animals as a supplement in the summer months. Since that hay could have been produced with commercial seed, it is possible that this plant-fungus association may have been artificially introduced in dehesa pastures.

Type I interactions in which all infected plants showed choke disease were observed in *Dactylis glomerata*, *Holcus lanatus* and *Alopecurus arundinaceus*. All flowering plants of these species which were examined were uninfected. The incidence of infections in these species was low. Infected *H. lanatus* and *A. arundinaceus* were found in only one location, while choked *D. glomerata* plants infected by *E. typhina* occurred in 5 locations. Since the sampling in those species was biassed towards collecting infected individuals showing choke disease, the percentage of infected plants which can be deduced from Table 1 is not a measure of the infection level in populations of these species, which actually is very low.

Table 1. List of grass species analyzed for the presence of endophytic mycelium

Grass species	No. infected plants/total analyzed	Grass species	No. infected plants/total analyzed
Aegilops ventricosa	0/2	*Elymus caninus*	0/2
Agrostis castellana	0/25	*Elymus pungens*	0/5
Agrostis pourretii	0/2	*Festuca arundinacea**	105/157
Agrostis spp.	0/5	*Festuca rubra* s.l.*	125/175
*Alopecurus arundinaceus**	5/25	*Gaudinia fragilis*	0/11
Alopecurus geniculatus	0/7	*Glyceria declinata*	0/9
Anthoxhantum odoratum	0/3	*Holcus lanatus**	2/9
Arrhenatherum album	0/3	*Holcus mollis*	0/2
Arrhenatherum elatius	0/4	*Hordeum murinum*	0/2
Avena sterilis	0/2	*Lolium perenne**	4/21
Briza maxima	0/5	*Phleum pratense*	0/8
Bromus catarticus	0/7	*Poa bulbosa*	0/32
Bromus commutatus	0/6	*Poa pratensis*	0/2
Bromus diandrus	0/1	*Poa trivialis*	0/4
Bromus hordaceus	0/7	*Poa* spp.	0/9
Cynosurus cristatus	0/8	*Polypogon monspeliensis*	0/1
Cynosurus echinatus	0/4	*Stipa lagascae*	0/6
*Dactylis glomerata**	17/31	*Vulpia membranacea*	0/1
Deschampsia media	0/3	*Vulpia myuros*	0/2
Echinochloa crus-galli	0/3	*Vulpia bromoides*	0/10

* Species which were found to host fungal endophytes

It is known that endophyte infected plants of *F. rubra, F. arundinacea*, and *L. perenne* contain several types of alkaloids which are toxic to herbivores (Siegel *et al.*, 1987). Therefore the presence of high rates of endophytic infection in populations of these species present in dehesa grasslands should be considered as an antinutritional factor which may affect pasture quality.

The rates of endophytic infection observed in plants of *F. rubra* s.l. and *F. arundinacea* were very high. Since endophytic infection in the genus *Festuca* can be advantageous for the host plants, it is possible that selective pressure in the form of drought stress and interspecific plant competition may favour those plants which are infected by endophytes in dehesa grasslands.

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