The development and application of ELISA detection of *Lolium* endophyte in ryegrass staggers research

D. R. MUSGRAVE,

Plant Diseases Division
Department of Scientific and Industrial Research, Lincoln

L. R. FLETCHER

Grasslands Division
Department of Scientific and Industrial Research, Lincoln

ABSTRACT

An enzyme-linked immunosorbent assay (ELISA) system has been developed for the detection of an endophytic fungus of *Lolium perenne*. The technique has been used to demonstrate quantitative differences in the spatial and tissue distribution of the endophyte in vegetative and reproductive tillers of infected plants. The influence of this on pasture toxicity is discussed.

The nature of the endophyte antigen is described and the relationship between the antigen and toxicity discussed.

Keywords ELISA; *Lolium* endophyte; *Lolium perenne* L.

INTRODUCTION

An enzyme-linked immunosorbent assay (ELISA) system has been developed for the detection of an endophytic fungus of *Lolium perenne* L. (Musgrave, 1984). This endophyte has been identified as *Acremonium* sp. (G. C. M. Latch, pers. comm). ELISA has been used to determine the concentration of endophyte mycelium in particular ryegrass tissues and the percentage of plants and seed infected. The correlation between the presence of an endophyte and clinical symptoms of ryegrass staggers has been well documented (Fletcher and Harvey, 1981; Mortimer *et al.*, 1982; Keogh, 1973; 1978) has suggested that grazing the basal portion of ryegrass pastures is a prerequisite for the onset of clinical ryegrass staggers. Use of ELISA has shown that *Acremonium* sp. endophyte is concentrated in the basal part of the vegetative plant (Musgrave, 1984). Fletcher (1983), has suggested that depression of live-weight gains due to the presence of *Acremonium* sp. endophyte may occur even in the absence of clinical ryegrass staggers.

METHODS

Twenty-five individual 'Ellett' ryegrass plants from Canterbury pasture, were separated into vegetative and reproductive tillers and sectioned as described by Musgrave (1984), except that cuts were made at 0, 5, 15, 25, 35 cm for reproductive tillers and 0, 5 and

TABLE 1 Distribution of *Acremonium* sp. endophyte in sections of vegetative and reproductive tillers of *Lolium perenne* L.[1]

Plant Section[3]	% of total endophyte mycelium		Concentration of endophyte mycelium[2]	
	Vegetative tiller	Reproductive tiller	Vegetative tiller	Reproductive tiller
-10-0	1.4	1.4	6.4	4.5
0-5	75.1	38.3	40.9	21.9
5-15	21.5	30.3	20.9	16.3
15-25	2.0	10.5	0.9	4.5
25-35	–	8.3	–	4.7
35-45	–	11.2	–	5.5

[1] Results are the average of 3 replicates.
[2] μg mycelium fresh weight/mg plant material dry weight.
[3] Height (cm) relative to ground level (0).

15 cm for vegetative tillers. Vegetative tillers had an average height of 28.15 ± 4.57 cm and reproductive tillers an average height of 44.58 ± 6.75 cm.

All samples were dried and 60 mg was resuspended in 3.0 ml of sample buffer. Triplicate samples were analysed by ELISA using the method of Musgrave (1984).

RESULTS

In vegetative tillers 76.6% of the total endophyte mycelium in the plant was below 5 cm and in reproductive tillers only 39.7% was in this section (Table 1). Although the average concentrations in the whole tillers are not significantly different, the concentration of endophyte mycelium, which is highest in both tillers in the 0 to 5 cm section, decreases more with height in vegetative than reproductive tillers.

In vegetative tillers the concentration in the leaf sheath was significantly higher than any of the other tissues (Table 2). In the reproductive tillers the concentration in the crown, flowering stem and leaf sheath was not significantly different. Approximately half of the endophyte mycelium was found in the leaf sheath of vegetative tillers whereas greater than two-thirds of the mycelium was found in the flowering stem of reproductive tillers.

TABLE 2 Distribution of *Acremonium* sp. endophyte in tissues of vegetative and reproductive tillers of *Lolium perenne* L.[1]

Plant tissue	% of total endophyte mycelium		Concentration of endophyte mycelium[2]	
	Vegetative tiller	Reproductive tiller	Vegetative tiller	Reproductive tiller
Roots	1.4	1.4	6.4	4.5
Crown	17.8	6.3	23.8	17.9
Flowering stem	–	69.0	–	12.7
Pseudostem	35.2	–	25.7	–
Leaf sheath	43.6	21.4	44.6	13.6
Leaf	2.0	1.9	0.9	2.8

[1] Results are the average of 3 replicates.
[2] *μg mycelium fresh weight/mg material dry weight.*

The *Acremonium* sp. antigen has been shown to be a high molecular weight (> 150 000 d) polysaccharide moiety of a lipopolysaccharide-protein complex (LPS) found as a surface component of endophyte mycelium (D. R. Musgrave *et al.*, pers. comm.). The LPS antigen can be purified to homogeneity from liquid culture filtrates of *Acremonium* sp. cultures or from the mycelium itself. The soluble antigen is identical in antigenicity and biochemical properties to that solubilised from intact mycelium.

DISCUSSION

The differences in the spatial and tissue distribution of the *Acremenium* sp. endophyte within the ryegrass plant is seen to reflect the transition from the vegetative to the reproductive phase. In the vegetative tillers the endophyte is found predominantly in the leaf sheath of the 0 to 5 cm section. This contrasts with the reproductive tiller in which the endophyte is found predominantly in the flowering stem with similar endophyte concentrations in crown, leaf sheath and flowering stem. The significance of this is that with reproductive tillers animals would not have to graze the base of the pasture for endophyte effects to occur.

Acremonium sp. endophyte concentrations of approximately 5 μg/ml or greater are found in all plant sections except the uppermost vegetative section which consist solely of leaf. We therefore suggest that changes in the distribution of endophyte outlined in this paper may lead to significant animal effects. There is evidence (L. R. Fletcher, pers. comm.) which shows that live-weight gains of hoggets can be lower on *Acremonium* sp. containing pasture even in the absence of ryegrass staggers.

The LPS nature of the *Acremonium* sp. antigen suggests that the ELISA system measures a major component of the fungal cell wall. If it is assumed that a fungal product is the toxin involved in ryegrass staggers then ELISA measurement of fungal mycelium would predict the potential for toxicity and not the toxicity *per se*. Therefore it would be wise in future studies to measure both the amount of fungal mycelium and the toxicity of plant samples.

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