THE **LOLIUM** ENDOPHYTE: RETURN FROM ANONYMITY

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INTRODUCTION

The commensal, above ground fungus in ryegrass, known simply as the *Lolium* endophyte, which has been well studied in the past (McLennan, 1920; Sampson, 1935, 1937; Neil, 1940, 1941; Lloyd, 1959) has recently emerged as the bright new hope in the ryegrass stagger aetiology research. Fletcher and Harvey (1981) showed a highly significant correlation between the amount of infection in three ryegrass cultivars and the observed intensity of ryegrass staggers in a split plot randomised grazing trial at Lincoln. At the same time, a new group of neurotoxins, the lolitrems, were also being linked with ryegrass staggers by Gallagher, et. al (1981). It would appear that an association between the endophyte and the host trigger the production of these lolitrems, but whether they are produced by the host in response to invasion of the intercellular space by the fungus (as is the case in the production of phytoalexins) or by the fungus itself in response to specific apoplastic nutrients supplied by the host, has not been fully established.

Ironically, the *Lolium* endophyte was suggested the possible aetiological agent in ryegrass staggers several years ago. It was rejected, however, on three counts: Firstly, when axenic cultures of the fungus (grown on ryecorn seed) were fed to sparrows and mice, no ill effects were noted (Cunningham, 1958). Secondly, when endophyte-infected seed was fed to a range of animals, including one sheep, no toxicity was noted (Neil, 1941); and thirdly, it was considered by Neil in 1940 that "a widely distributed fungus such as *Lolium* endophyte could not be responsible for localised outbreaks of ryegrass staggers!" (Cunningham & Hartley, 1959).

The Lincoln group of researchers have used two approaches to determine why these three factors caused such a rejection. Firstly, an ultrastructural study was carried out at Canterbury University on the interaction between the fungus and the host, and the nature of the fungus in seed and in pure culture (Fineran *et al*, in prep.; Gallagher *et al*. 1982). Secondly, a study of endophyte levels in some South Island pastures where outbreaks of ryegrass staggers had occurred was carried out (Harvey, 1982).

ULTRASTRUCTURAL STUDIES

Fineran, *et al* (in press) found unusual crystalloids and aggregates of tubules in cytoplasm of the *Lolium* endophyte growing in ryegrass leaf sheaths. These structures were particularly noteworthy because of the comparatively large amount of cytoplasm they occupied, and their apparently unique structure. Crystalloids were noted in the basal portion only in flowering stems of endophyte-injected plants, not in the upper portion although tubule aggregates were readily seen.

In seeds, the cytoplasm was dense and lacked detail, being typical of mycelium that had become dormant. Similar cytoplasmic details were discovered in over-

wintering mycelium in ryegrass leaf sheaths, collected from the field in early August.

One line of fresh seed however, harvested before maturity and artificially dried, contained mycelium that was not fully dormant, and crystalloid remnants were detected although no tubule aggregates could be seen. Seeds from the same line, fixed three months later had fully dormant mycelium, and no detectable crystalloids.

A pure culture of the endophyte, grown on potato dextrose agar, was completely devoid of crystalloids and tubule aggregates, but contained typical fungal cytoplasmic organelles.

The question arises, are the unusual structures in the endophyte cytoplasm in any way connected with neurotoxin production? Neurotoxins (lolitrems) have been detected in endophyte infected ryegrass (Mortimer et. al, 1982), infected seed (Gallagher et. al, 1982), and infected flowering stems, but not in cultures of the fungus (R.T.Gallagher, pers. comm.). More research is underway to determine if the crystalloids or tubule aggregates have any direct relation to the accumulation or synthesis of neurotoxins. There is some evidence that they may play a part but more substantial proof is required. If however, the neurotoxins are produced by the host in response to invasion of the intercellular space by the endophyte, then other parasites or pathogens should be able to trigger a similar response, which, in plants, is often non-specific to invading organisms.

Table 1: INFECTION LEVEL OF THE *LOLIUM* ENDOPHYTE IN SOME SOUTH ISLAND PASTURES IN RELATION TO RYEGRASS STAGGERS

	None	Ryegrass Staggers Observed		
		Slight	Medium	Severe
No. of paddocks	6	4	13	7
Average Infection (0-5 Scale)	1.4	1.7	2.1	2.2
SE	1.4	1.04	0.63	0.44
Infection Range	0-3.8	0.9-3.1	0.9-3.2	1.5-2.8

A FIELD STUDY OF RYEGRASS PASTURES

This field study required the assistance of MAF field advisory officers, sheep and beef officers, researchers and veterinarians who sent plant samples from 30 paddocks from around the South Island to the Plant Health Diagnostic Station at Lincoln for *Lolium* endophyte analysis. In all cases where staggers occurred, the endophyte was present (Table 1). Where no staggers occurred, endophyte levels ranged from nil to moderately high. Thus, it would appear that more factors than just the presence of endophyte are required to precipitate the condition. These factors, from the survey, appeared to be —

- the proportion of ryegrass in the pasture;
- the height of the pasture;
- the age, condition, and species of the grazing animals

CONCLUSIONS

The reasons for the historical rejection of the *Lolium* endophyte as the cause of, or trigger factor for, ryegrass staggers are clearer with this recent research. If the unusual structures in the cytoplasm of the *Lolium* endophyte in the plant are linked with neurotoxin production, then their absence from cultures may explain the non-toxicity of the cultures and their lack of lolitremes. The non-toxicity of infected seed is not so easily explained. Gallagher, et. al (1982) found that not all of the sheep they fed with pellets made from infected ryegrass seed produced symptoms of ryegrass staggers. The earlier feeding trial reported here included only a single sheep among the trial animals, and this sheep may have been non-reactive to the neurotoxins in the seed.

Factors other than the presence of the *Lolium* endophyte can contribute to an outbreak of ryegrass staggers. These factors include -the height of the pasture (the fungus is found in higher concentration in leaf sheaths than in leaves); the proportion of ryegrass in the sward; the level of neurotoxin in the forage bulk ingested; and the species of animal and pattern of its grazing.

The *Lolium* endophyte has finally gained prominence, not only because of its implication in the aetiology of ryegrass staggers, but also because of the property of infected ryegrass plants to resist attack by the important pasture pest, Argentine stem weevil (*Listronotus bonariensis*) (Prestige et al, 1982). It is interesting to note that a fungus of such import as the *Lolium* endophyte has yet to be assigned a Latin binomial.

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