ENEFICIAL USE OF GRASSES ARTIFICIALLY INFECTED WITH ENDOPHYTES, NON-TOXIC TO ANIMALS

G.C.M. Latch and L.R. Fletcher
AgResearch, Palmerston North, New Zealand

ABSTRACT
Information presented in this paper demonstrates that Ryegrass Staggers does not occur in animals which graze ryegrasses (*Lolium sp.*) artificially infected with a strain of *Acremonium* endophyte which does not produce the tremorgenic toxin lolitrem B. Peramine is produced by the endophyte and this alkaloid protects the grass from attack by Argentine stem weevil. New Zealand farmers have used a ryegrass cultivar infected with this strain of endophyte for the past four years and there are no reports of Ryegrass Staggers in animals grazing it. Strains of endophytes have been found in other grasses which do not produce toxins harmful to animals. It is likely that grasses infected with these strains may have improved growth and persistence and superior animal production compared with grasses which are endophyte-free or infected with non-selected endophytes.

KEYWORDS
Endophyte, fungal strains, Ryegrass Staggers, lolitrem B, Argentine stem weevil, peramine

INTRODUCTION
Seedborne fungal endophytes are present in many temperate grasses. Mycelium is intercellular and concentrated in leaf sheaths, culms and seeds. Grasses infected with *Acremonium* endophytes are symptomless but many compounds are produced by the endophyte which affect grass growth and persistence and the health of grazing animals.

Research in New Zealand and the USA has shown the desirability of endophyte-infected grasses in pastures because the endophyte protects the grass against biotic and abiotic stresses (Bacon, 1993; Latch, 1993). *Acremonium* endophytes in perennial ryegrass (*Lolium perenne*) produce peramine which protects the grass from attack by Argentine stem weevil (*Listronotus bonariensis*), a serious pest of New Zealand pastures (Rowan et al.,.1990). However, lolitrem B the cause of Ryegrass Staggers is also produced (Gallagher et al., 1984).

Strains of *Acremonium* have been found which produce peramine but not lolitrem B. This paper describes the successful introduction of one such strain into a cultivar of ryegrass, its release to farmers and the elimination of Ryegrass Staggers in animals which graze pastures sown with this cultivar.

MATERIALS AND METHODS
Endophytes. Perennial ryegrass seeds were collected from Europe, North Africa, Australia and New Zealand. Thirty seeds from each collection were squashed, stained and examined with a microscope for the presence of endophyte mycelium. Seeds from infected samples were sown in a greenhouse and infected plants detected by examining the leaf sheaths for mycelium. Leaf sheaths from infected plants were harvested, freeze dried and analysed by HPLC for the alkaloids lolitrem and peramine.

Plant infection. Endophytes were cultured from those plants which produced peramine but not lolitrem. One of these endophyte strains (187BB) was inoculated into seedlings of the hybrid ryegrass cultivar Greenstone (*L. x boucheanum*) and the perennial ryegrass cultivars Ruanui, Nui and Pacific using the technique of Latch and Christensen (1985). A second strain of endophyte (Waiau) which produced large amounts of lolitrem and peramine in its host plants was also inoculated into seedlings of all these cultivars except Pacific. Seed was collected from the infected plants and sown for further multiplication.

Grazing trial. Plots measuring 126m x 7m were established with seeds from the cultivars artificially infected with the two strains of endophytes. Plots were also sown with seeds of Nui and Pacific infected with their normal endophytes. Endophyte-free seed of the four cultivars was sown in plots to act as controls. When the grass was well established 9 lambs were put on each plot for 28 days and their condition monitored. Numbers of Argentine stem weevils in plots and the damage they did to the grass was also monitored.

RESULTS
The strain of endophyte in the ryegrass had a significant effect on the incidence of Ryegrass Staggers and liveweight of lambs. Animals grazing ryegrasses infected with 187BB or endophyte-free ryegrasses showed little or no symptoms of Ryegrass Staggers whereas lambs grazing ryegrasses infected with Waiau or the strains normally present in the cultivars showed severe staggers. Weight gain followed a similar pattern. (Fletcher et al., 1991).

Argentine stem weevil damage was significantly greater in endophyte-free plots and there were nearly twice as many larvae in these plots compared with those in plots sown with endophyte-infected ryegrasses.

Levels of lolitrem B averaged 8.0mg/ml in Waiau-infected ryegrasses, 4.7mg/ml in ryegrasses infected with their normal endophyte, 0.1mg/ml in ryegrasses infected with 187BB and 0 in endophyte-free ryegrasses. Peramine levels ranged from 11-18mg/ml in the endophyte-infected ryegrasses and 0 in the endophyte-free ryegrasses.

DISCUSSION
This trial showed that lambs grazing ryegrasses infected with a strain of endophyte which did not produce lolitrem B were generally free from Ryegrass Staggers. Very mild staggers were observed in lambs grazing Ruanui infected with 187BB and it is possible that the combination of this cultivar and the endophyte produced a small amount of another toxic compound. All endophyte-infected cultivars produced sufficient peramine to reduce damage from Argentine stem weevil.

Larger and and more extensive animal trials have confirmed the results obtained in this initial trial. Greenstone infected with the 187BB strain of endophyte has been released commercially and is used widely by farmers in New Zealand. Over the four years that this cultivar has been available no reports of Ryegrass Staggers have been brought to our attention and pastures have persisted indicating that the ryegrass was resistant to Argentine stem weevil.

The endophyte 187BB produces ergovaline and it is believed that this alkaloid is the cause of health problems when animals eat tall fescue infected with *A. coenophialum* (Porter, 1994). Further strains of endophytes from ryegrass have been identified which produce neither lolitrem B nor ergovaline and these strains have been

artifically infected into several cultivars of ryegrasses. Animal feeding trials will commence shortly with these strains to determine whether they are an improvement on strain 187BB.

Thus the principle has been established that it is possible to reduce or eliminate the deleterious effects that endophytes have on animals while preserving the beneficial effects they have on the grass host. Strains of *A. coenophialum* have been found which do not produce ergovaline so it may be possible to overcome some of the health problems that animals suffer when they eat endophyte-infected tall fescue. However, where the endophyte appears to offer no advantage to grass growth and persistence it may be desirable just to eliminate the endophyte from the grass.

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