AN ASSOCIATION OF *LOLIUM* ENDOPHYTE WITH RYEGRASS RESISTANCE TO ARGENTINE STEM WEEVIL

R.A. PRESTIDGE, R.P. POTTINGER, G.M. BARKER

Ruakura Soil & Plant Research Station, MAF, Private Bag, Hamilton

SUMMARY

Previous results have shown a large degree of variability in resistance/tolerance within and between cultivars of perennial ryegrass (*Lolium perenne*) to Argentine stem weevil (*Listronotus bonariensis*). Much of this variability may be explained by the presence of an endophytic fungus (*Lolium* endophyte) present in perennial and hybrid ryegrasses. This paper presents evidence to show that the presence of the endophyte confers resistance to *Lolium* against stem weevil attack.

INTRODUCTION

Argentine stem weevil is an important pest of Gramineae in New Zealand. The larvae enter and mine the interior of the stem, eventually destroying the crown and killing the tiller. There are generally two generations each year, with oviposition commencing in early spring and ceasing in late autumn when the adults overwinter in a state of reproductive diapause (Goldson 1981).

Species and cultivar differences exist among pasture grasses in weevil oviposition preferences and larval damage tolerances (Pottinger 1961; Kain *et al* 1977, 1982; Goldson 1982; Barker and Pottinger 1982; Gaynor and Hunt 1982). These studies have shown that annual ryegrasses (*Lolium multiflorum*) are much more susceptible to Argentine stem weevil attack than perennial ryegrasses. Considerable variability is apparent in the results of studies on interactions between Argentine stem weevil and cultivars of perennial and hybrid ryegrasses, e.g. contradictory evidence on the relative susceptibility to stem weevil of the cultivars 'Grasslands Ariki', 'Grasslands Nui', 'Grasslands Ruanui' and 'Ellett' has come from studies within and between different regions, namely Canterbury (Goldson 1979a, 1982), Manawatu (Kain *et al* 1977, 1982), and Waikato (Barker and Pottinger 1981), and under different nutritional and environmental conditions (Gaynor and Hunt 1982).

The mechanisms involved in Argentine stem weevil-ryegrass interactions are not yet understood, but an apparent effect of host infection by an endophyte on resistance of ryegrass to Argentine stem weevil was recently observed. Most lines of perennial ryegrass in NZ carry an endophytic fungus as intracellular hyphae penetrating aerial tissues (Neill 1941; Lloyd 1959).

In an experiment to investigate the animal disease "ryegrass staggers" four seed lines of Ellett ryegrass were sown in spring 1981 at Ruakura. Two lines had endophyte levels of less than 3% and two had endophyte levels greater than 90%. The low endophyte plots were found to contain five times as many Argentine stem weevil adults and suffered much greater pasture damage than the high endophyte plots in March 1982 (Mortimer *et al* 1982). This paper reports further evidence of the effects of endophyte on resistance among ryegrasses to Argentine stem weevil.

METHODS

A trial was established at Rukuhia on a Horotiu sandy loam in autumn 1981 for the study of population dynamics and damage by stem weevil in four ryegrass cultivars (Table 1) sown with 'Grasslands Huia' white clover (*Trifolium repens*). Six replicates of 8 x 50 m plots were broadcast with ryegrass seed at 25 kg/ha and white clover at 2 kg/ha. Plots were rotationally grazed with sheep at approximately monthly intervals.

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Cultivar performance was assessed by yield and tiller measurements in replicated untreated and insecticide protected areas. Applications of oxamyl (480 g/ha) at 10-15 day intervals from August to April maintained the latter areas relatively free of insect infestation. From the untreated areas, Argentine stem weevil adult populations were assessed fortnightly by taking twenty 7.5 cm diameter soil cores per plot and extracting the adults by a wet sieving and flotation technique (Barker unpublished). Larval populations were assessed fortnightly by cutting tillers at ground level at 10-15 randomly selected points per plot and hand counting 200 ryegrass tillers from each plot. Larvae were heat extracted by the method of Goldson (1978).

RESULTS AND DISCUSSION

There were marked differences in infestation levels and production losses among the cultivars (Table 1). The second generation population was greater than the first generation which is typical of Waikato conditions (Barker and Pottinger 1982). Ellett ryegrass, with its significantly smaller ($p < 0.01$) larval and adult populations, suffered considerable tiller losses, but survived relatively well when compared to losses in the hybrid ryegrass, 'Grasslands Manawa'.

If endophyte host infection has an adverse effect on Argentine stem weevil, the insects should feed selectively from plants that are endophyte free. Therefore untreated pastures should contain a higher proportion of endophyte infected plants than insecticide protected areas after a period of infestation. At the end of the first year of Argentine stem weevil infestation, frequencies of endophyte-infected and endophyte-free plants were estimated by microscopic examination of tillers (Table 2). There were significantly more endophyte-infected plants in the untreated areas on all cultivars ($p < 0.01$). Furthermore there was a good correlation between endophyte levels and weevil numbers (Fig. 1) and with ryegrass tiller losses across the four cultivars.

TABLE 1: Argentine stem weevil numbers, corresponding tiller numbers and percentage tiller loss due to stem weevil attack.

Ryegrass Cultivar*	Stem weevil No./m ²				Live tillers/m ²		% tiller loss
	Peak larval No.	Peak adult No.			February 1982		
	1st generation	2nd generation	1st generation	2nd generation	U	I	
Manawa	62	436	206	223	250	1680	85
Nui	38	510	96	217	2030	4340	53
Ruanui	35	464	77	223	3170	4950	41
Ellett	18	125	32	55	3860	5540	30

* Manawa = *L. multiflorum* x *L. perenne*. Nui, Ruanui and Ellett = *L. perenne*.

U = untreated

I = insecticide protected

TABLE 2: Mean endophyte infections in 11 month old ryegrass pastures, with and without insecticide protection (March 1982). Sixty tillers examined per treatment.

Ryegrass cultivar	Percent plants with endophyte	
	Untreated	Insecticide
Manawa	10.0 c	8.3 c
Nui	41.7 b	23.3 b
Ruanui	58.3 b	35.0 b
Ellett	83.3 a	66.7 a
Mean	48.3	33.3

Fig. 1: Relationship between endophyte infection and second generation adult stem weevil populations. (● this study; x Mortimer *et al* 1982). ($y = -1.98X + 254.8$, $r = 0.85^*$).

These initial observations suggest some adverse effect of endophyte on Argentine stem weevil or preference for endophyte-free plants. It raises many questions on the true nature of resistance to Argentine stem weevil among ryegrasses and among other grass species which are also known to contain specific endophytes.

From knowledge of *Lolium* endophyte and Argentine stem weevil several analogies can be drawn. The endophyte is specific to *L. perenne* and is not known for *L. multiflorum*. Argentine stem weevil larval damage is particularly severe in *L. multiflorum* with 80-100% tiller kill in a single season (Pottinger 1961; Barker and Pottinger 1982). Damage in perennial ryegrasses is generally between 5-50% tiller kill (Table 1, Pottinger 1961, Kain *et al* 1977, Barker and Pottinger 1982). Endophyte infection is transmitted from one plant generation to the next in the seed and high endophyte infection is typical of most older pastures (Lloyd 1959). Weevil numbers and damage levels gradually decline with aging of the pasture (Barker and Pottinger, unpublished data), presumably by *in vivo* selection of resistant plants. Seed lines contain a mixture of endophyte free and endophyte infected seed with infection decreasing with increasing seed age (Lloyd 1959). This may partly explain the variations

in weevil numbers and damage within and between perennial and hybrid cultivars observed in different studies and why individual plants within cultivars are readily categorised as either weevil resistant or not (Kain *et al* 1982).

The endophyte of *Lolium* is not pathogenic to its host (Neill 1941) and the association seems commensal in character. The observations indicating the presence of mammalian neurotoxins and the other associated compounds (Gallagher *et al* 1981) raises the question of the functions of the endophyte and the toxins in ryegrasses. It suggests that they are antiherbivore materials, similar in function to compounds often found to impart resistance to dicotyledons against herbivorous insect attack.

If steps are taken to eliminate endophytic fungi from ryegrasses to effect ryegrass staggers control, this could have serious and devastating implications in regard to Argentine stem weevil damage in pastures throughout New Zealand. Reduction in endophyte infection levels could result in high loss of ryegrasses from our swards. Until we have nonendophyte stem weevil resistant perennial ryegrass strains, it would be foolhardy to breed ryegrass staggers resistant cultivars.

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