Proceedings of the New Zealand Grassland Association 44: 267-263 (1983)

THE RELATIONSHIP BETWEEN NITROGEN SUPPLY, ENDOPHYTIC FUNGUS, AND ARGENTINE STEM WEEVIL RESISTANCE IN RYEGRASSES

D.L.GAYNOR and W.F.HUNT
Entomology Division and Grasslands Division, DSIR, Palmerston North.

Abstract

Experiments are described which indicate that the degree to which ryegrasses, *Lolium* spp., are damaged by Argentine stem weevil, *Listronotusbonariensis* (Kuschel), is affected bv three major factors. Ryegrasses with high levels of infection with endophytic fungi carry fewer *stem* weevil eggs and larvae and consequently suffer less damage. Some ryegrasses with low levels of endophyte infection, such as 'Grasslands Ruanui', suffer more damage when *grown* with high inputs of nitrogen, while others with the genotype *Lolium multiflorum x perenne* L., such as 'Grasslands Manawa' are more susceptible than those with the genotype *Lolium perenne* L.

Keywords: Lolium spp., Listronotus bonariensis, endophvtic fungus, nitrogen, plant resistance, stem weevil

INTRODUCTION

Argentine stem weevil, *Listronotus bonariensis* (Kuschel), is a serious pest of ryegrasses throughout most of New Zealand. Stem weevil larvae tunnel inside the stem of the ryegrass *(Lolium* spp.) tiller and eventually kill it. In severe infestations enough tillers can be killed to result in the death of the whole plant. The insect has two and occasionally three generations a year, the main peaks in the larval population occurring in October and January-February. Adult weevils feed on the tips of the ryegrass leaves, eating through the leaf to the lower epidermis leaving behind small rectangular "windows" in the leaf. The adults do not normally affect the plant significantly but can kill very young seedlings.

The study of the effects of stem weevil on different types of ryegrasses has produced confusing and often conflicting results. 'Grasslands Nui' perennial ryegrass *(L. perenne* L.) has been found to suffer more damage than 'Grasslands Ruanui' (Kain et al. 1977), but in other experiments no differences between the two cultivars were detected (Goldson 1979a, Kain et al. 1982), while in others Ruanui grown with high inputs of nitrogen and water was more susceptible than Nui (Hunt & Gaynor, 1982). Conflicting results have also been found with 'Grasslands Ariki' ryegrass (Goldson 1979a,b, Kain et al. 1977, Lancashire et al. 1977).

Other ryegrasses such as an ecotype selection from Takapau, Hawke's Bay and 'Ellett' ryegrass appear to be resistant to stem weevil (Kain et al. 1982). Recently, however, Prestige et al. (1982) have indicated that stem weevil resistance is associated with the presence of an endophytic fungus in the tissues of the resistant plants. Since 1978 we have been investigating the effects of stem weevil on different ryegrass cultivars and seed lines, particularly under low and high rates of nitrogen application. The levels of endophyte infection have been determined for each experiment and are compared below with a summary of stem weevil effects (the details of which have or are being published elsewhere).

Table 1: ENDOPHYTE INFECTION LEVELS (MICROSCOPE 7/5/82; ELISA* 5/10/82), RYEGRASS-ONLY YIELDS (23/2/82), AND ARGENTINE STEM WEEVIL DAMAGE († CUT SAMPLES 12/1/82, ‡ TAGGED TILLERS 12/12/81 – 23/2/82) FOR FIVE PERENNIAL RYEGRASSES.

Ryegrass Line	Endophyte Microscope	ELISA Index	Eggs Per 100 Tillers †	Larvae Per 100 Tillers †	Damaged Tillers % †	Larval Damage % ‡	Mean Yield (kg DM/ha)
Nui	20a	1.39 a	22.6 a	5.8 a	17.0 a	74 a	1435 b
Selection	15a	2.25 a	11.8 b	3.7 b	12.8 a	56 b	667 c
Takapau	98 b	5.09 c	1.8 c	1.4 c	1.7 b	30 c	865 bc
Low C. Ariki	93 b	3.93 b	3.8 c	0.9 c	1.8 b	26 cd	2165a
Ellett	94 b	5.13 c	4.2 c	1.0 c	2.3 b	18d	1509 ab

a,b,c,d -values followed by the same letter are not different at $p<0.05$

*ELISA index – Enzyme-linked immunosorbent assay – values below 1.1 are considered as not containing any endophyte.

(notes apply to Tables 2 and 3)

The results indicate that the severity of stem weevil attack is affected by three main factors: endophyte, nitrogen and genotype. These effects are discussed separately.

ENDOPHYTE EFFECT

In 1981 a field trial was sown to compare the effects of stem weevil on five lines of perennial ryegrass: Nui, Ellett, a low cellulose selection of Ariki, an ecotype selection from Takapau, Hawke's Bay and a selection based on plants showing lower levels of adult stem weevil damage (referred to as 'Selection'). The Ellett and Takapau ryegrasses were reported by Kain et al. (1982) to be resistant to stem weevil. The experimental technique using tagged tillers was the same as described in Hunt and Gaynor (1982) but yield cuts were taken at the end of regrowth periods. Treatments were replicated three times and plots were 2.3 x 5 m.

Ten tillers from each plot were examined microscopically for the presence of endophyte, to indicate the proportion of plants infected. In addition, twenty tillers from each plot were bulked together and tested using the enzyme-linked immunosorbent assay (ELISA) (Clark & Adams 1977) to indicate the quantity of endophyte present in plants (ELISA index). This is regarded as a more sensitive detection method (D.Musgrave pers. comm.). The results are presented in Table 1.

The endophyte levels were significantly higher for Takapau, Ellett and low cellulose Ariki than for Nui and Selection. The egg numbers, larval numbers, and percent damaged tillers both from the cut samples and the tagged tiller examinations were significantly lower for Takapau, Ellett and low cellulose Ariki than for Nui and Selection. The ryegrass yields taken at the end of the main stem weevil period showed that Ariki yielded more than Nui, Takapau and Selection, and Nui and Ellett yielded more than Selection. It is possible that yield differences in part reflected-differences in stem weevil damage.

The trial has clearly shown that where endophyte levels were high, stem weevil damage was low. The low egg lay in the endophyte-infected ryegrasses points to an effect probably associated with adult weevil behaviour.

NITROGEN EFFECT

In 1978 a field trial set up to examine the effects of high nitrogen inputs on differences- in growth in Ruanui and Nui ryegrasses was examined for stem weevil damage. Each of the two cultivars received two nitrogen treatments: nitrogen irrigated onto the plots at rates twice the anticipated growth requirement to allow for a 50% loss between applications, or no added nitrogen or water. The 4 x 4 m plots were replicated 4 times and the details of the experimental technique are given in Hunt & Gaynor (1982). The trial was maintained until 4 March 1981 when samples were taken, frozen and later examined for endophyte. The results are presented in Table 2.

The proportion of Ruanui plants with endophyte was significantly lower than in Nui when examined microscopically but the more sensitive ELISA test showed that endophyte levels were low with no significant differences between the treatments. Larval numbers were significantly higher in Ruanui than in Nui and both cut samples and tagged tiller examinations showed that only the Ruanui high nitrogen treatment suffered severe stem weevil damage. This was re-

Table 2: ENDOPHYTE INFECTION LEVELS (MICROSCOPE; ELISA 4/3/81) GROWTH RATE (12/1/79 – 20/2/79), AND ARGENTINE STEM WEEVIL DAMAGE († CUT SAMPLES 5/1/81, ‡ TAGGED TILLERS 12/1/79 – 20/2/79), FOR NUI AND RUANUI PERENNIAL RYEGRASS GROWN WITH LOW (LN) AND HIGH (HN) NITROGEN INPUTS.

Cultivar	Nitrogen Level	Endophyte Microscope	Endophyte ELISA index	Larvae Per 100 Tillers †	Damaged Tillers % †	Larval Damage % ‡	Growth Rate (kg DM/ha/day)
Nui	LN	54 a	1.85 a	4.1 a	19.2 a	43 a	13 a
	HN	84 a	2.07 a	4.7 a	20.9 a	56 a	110 b
Ruanui	LN	25 b	1.81 a	7.2 b	25.8 a	46 a	9 a
	HN	42 b	1.58 a	8.4 b	41.4 b	82 b	20 a

Table 3: ENDOPHYTE INFECTION LEVELS (MICROSCOPE 24/2/81; ELISA 14/3/81), YIELD LOSS (23/2/82), AND ARGENTINE STEM WEEVIL DAMAGE († CUT SAMPLES 12/1/82, ‡ TAGGED TILLERS 1/2/80 – 7/3/81), FOR FOUR RYEGRASS CULTIVARS TREATED AND UNTREATED WITH OXAMYL.

Cultivar	Endophyte Microscope	Endophyte ELISA index	Eggs Per 100 Tillers †	Larvae Per 100 Tillers †	Damaged Tillers % †	Larval Damage % ‡	Yield Loss (kg DM/ha)
Unsprayed							
Manawa	0	1.27 a	19.5 a	10.5 a	40.3 a	72 a	1441
Ruanui	0	1.32 a	12.8 ab	7.8 ab	23.3 bc	19 c	-105ns
Nui	0	1.59 a	13.3 ab	9.5 a	27.0 b	47 b	337 ns
Ariki	0	1.40 a	19.3 a	4.3 bc	18.8 c	48 b	611 ns
Sprayed							
Manawa	0	1.42 a	10.8 ab	0.8 c	4.0 d	16 c	
Ruanui	0	1.69 a	2.5 b	0.0 c	1.0 d	4 c	
Nui	0	1.51 a	6.5 b	0.5 c	3.3 d	11 c	
Ariki	0	1.44 a	3.8 b	0.5 c	2.3 d	8 c	

ns – no significant difference between yields for sprayed and unsprayed.

– $p<0.05$ difference between yields for sprayed and unsprayed.

flected in the very low growth rate for Ruanui high nitrogen which in the absence of stem weevil damage was expected to be similar to the Nui high nitrogen treatment (Hunt & Mortimer 1982). Gaynor & Hunt (1982) reported that this was due to a preference by the adult weevils for plants grown with high nitrogen inputs, leading to high egg and larval numbers.

This trial has shown that in Ruanui ryegrass, with low levels of endophyte, high levels of nitrogen can markedly increase the amount of stem weevil damage.

GENOTYPE EFFECT

In 1979 a trial was sown which compared the effect of stem weevil on four ryegrass cultivars: 'Grasslands Manawa' *(L. multiflorum xperenne),* Nui, Ruanui and Ariki. The treatments were split to give an oxamyl sprayed (@ 1 kg a.i./ha) and unsprayed comparison. The experimental technique was the same as described in Hunt & Gaynor (1982) but did not include any nitrogen treatments. Tagged tillers were examined weekly and yield cuts were taken at the end of regrowth periods. The trial was maintained until April 1981 when samples were taken, froien and later examined for endophyte. The results are presented in "Table 3.

Microscope examination did not reveal any tillers infected with endophyte but the ELISA test showed that low levels of endophyte did exist with no significant differences between the treatments. Although egg and larval numbers were not higher in Manawa than in other treatments the amount of stem weevil damage was significantly higher. Spraying significantly reduced the stem weevil populations in all plots but the yield loss through not spraying was significant only in the Manawa treatment.

These results indicate that with low endophyte levels, short-rotation ryegrasses with a *L. multiflorum x perenne* genotype, such as Manawa, are more susceptible than perennial ryegrasses with a *L. perenne* genotype. The difference does not appear to be due to the presence or absence of endophyte.

CONCLUSION

The above results show that three major factors affect the degree to which a ryegrass cultivar will be damaged by stem weevil. A ryegrass which has a very high percentage of plants infected with endophyte will incur a lower egg lay and consequently suffer less damage. When endophyte levels are low then some cultivars, such as Ruanui, will suffer more damage from stem weevil when grown with high inputs of nitrogen. Also *L. multiflorum x perenne* ryegrasses will suffer more damage than *L. perenne* ryegrasses. Thus it appears that endophyte, nitrogen and genotype all affect the susceptibility of ryegrasses to stem weevil attack and these factors should be taken into consideration when growing ryegrasses in stem weevil prone areas.

ACKNOWLEDGEMENT

These experiments, which span five years, would not have been possible without technical support. We gratefully acknowledge the assistance of B.J. Mortimer, B.Holdaway, D.E.M.Calder, M.E.Hearfield, S.M.Olivecrona, P.J.Watts, and V.A. Hunt. We also acknowledge with thanks the assistance of M.J.Christiansen and D. Musgrave of Plant Diseases Division, DSIR, for the endophyte examinations.

REFERENCES

Clark, M.F.; Adams, A.N. (1977). *J.* Gen. *Vir. 34: 475-483*

Gaynor, D.L.; Hunt, W.F. (1982). *Proc. 3rd Aust. Conf. Grassld Invert. Ecol.,* (K.E.Lee, Ed) S.A. Govt. Printer, Adelaide: 255-263

Goldson, S.L. (1979a). The reproductive seasonality and morphology of Argentine Stem Weevil *Hyperodes bonariensis* Kuschel (Coleoptera: Curculionidae) and the effect of host grasses on its pre-reproductive development. Unpublished Ph.D. Thesis, Lincoln College, University of Canterbury, New Zealand. 250p.

Goldson, S.L. (1979b). *Proc. 2nd Aust. Conf. Grass/d Invert. Ecol.* (Crosby, T.K.; Pottinger, R.P. Eds.). Government Printer: Wellington: 262-264

Hunt, W.F.; Gaynor, D.L. (in press). *N.Z. J. agric. Res. 25:*

________; Mortimer, B.J. *(1982). Proc.* N.Z. *Grassld Ass. 43:* 125132

Kain, W.M.; Wyeth, T.K.; Gaynor, D.L.; Slay, M.W. (1982). N.Z. *J. agric. Res.* 25: 255-259

________' Ritchie, I.M.; Crouchley, C.G.; Smith, R.G.; Atkinson, D.S. (1977). *Proc. 3&h* N.Z. *Weed Pest Cont. Conf. : 192-I 97*

Kelsey, J.M. *(1958).* N.Z. *J. agric. Res. l: 790-795*

Lancashire, J.A.; Wilson, D.; Bailey, R.W. Ulyatt, M.J.; Singh, P. *(1977). Ibid* 20: 63-67

Pottinger, R.P. (1961). A study on the biology and economic importance of the Argentine Stem Weevil *Hyperodes bonariensis* Kuschel, in New Zealand. Unpublished M.Agr.Sci. Thesis, Lincoln College, University of Canterbury, New Zealand. 317p.

Prestige, R.A.; Pottinger, R.P.; Barker, G.M. (1982). *Proc. 35th* N.Z. *Weed Pest Cont. Conf.:* 119-122