

ARGENTINE STEM WEEVIL RESPONSE TO VARIABLE ENDOPHYTE INFECTION IN GRASSLANDS GREENSTONE RYEGRASS

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ABSTRACT

In three screenhouse trials three categories of endophyte infection of Grasslands Greenstone ryegrass plants were identified; viz. fully infected with the endophyte *Neotyphodium lolii*, partially infected with *N. lolii*, or completely *N. lolii*-free but infected with a *Gliocladium*-like endophyte. Damage by Argentine stem weevil larvae was higher on plants partially infected with *N. lolii* than on fully infected plants but in two trials was not as high as damage to *N. lolii*-free plants. Occurrence of partially infected plants appears to be variable and may be environmentally induced. The presence of the *Gliocladium*-like endophyte in some plants in one trial significantly increased larval damage to those plants, except where *N. lolii* was also present.

Keywords: *Neotyphodium lolii*, *Gliocladium*, ryegrass, insect damage, Argentine stem weevil

INTRODUCTION

Seed of the tetraploid hybrid ryegrass, Grasslands Greenstone (*Lolium multiflorum* x *perenne*) infected with an endophyte (*Neotyphodium lolii*) which does not produce lolitrem B, the causative agent of ryegrass staggers is commercially available. Despite the absence of lolitrem B, this endophyte confers a high degree of resistance to Argentine stem weevil (ASW) (*Listronotus bonariensis*) (Popay *et al.* 1995).

During previous studies of Greenstone, it was noted that the *N. lolii* endophyte could not be found in a small proportion of tillers on otherwise infected plants. A second *Gliocladium*-like endophyte was also found to occur naturally in some tillers. This endophyte occurred both in plants that were not infected and in plants that were infected with *N. lolii*.

The effect of this variable endophyte status on damage by ASW was investigated in three trials. This paper presents the results of these studies.

METHODS

Trial 1

In August 1992 seed trays containing potting mix were planted with Grasslands Greenstone seed. Throughout the trial these were kept outside on a concrete pad at Grasslands in Palmerston Nth. In early November 150 adult ASW were released onto each seed tray which was then covered with fine nylon net. The covers were removed after 3 weeks. On January 27, 1993, all plants were harvested by cutting at the base and a random selection of at least 10 tillers from each of 20 plants in each of five seed trays (100 plants in total) were subsequently checked for ASW adult feeding, larval damage and endophyte status. For adult feeding each tiller was given a feeding score of between 0 and 5 (0 = no feeding, 5 = extensive feeding scars over leaf blade). Tiller pseudostems were then stripped and examined for evidence of larval mining and a strip of epidermal sheath was taken for examination for endophyte.

Trial 2

Four polystyrene planter boxes containing commercial potting mix were each planted in September 1995 with 28 germinating seed of Grasslands Greenstone. Plants were kept in a screenhouse at Ruakura Research Centre. In early November, 50 field

collected ASW adults were released onto each planter box. The boxes were not covered. Two weeks later each plant was checked for adult feeding and eggs. Each plant was given a feeding score of 0 - 5 (see above) and egg numbers were recorded on the plant *in situ*. A further release of 50 weevils per box was made in mid January 1996. On April 9 all plants were cut at the base and stored in plastic bags at 4°C for a month to 6 weeks before they were examined for larval damage and the presence of endophyte. All tillers were examined for larval damage and an epidermal strip was taken from a random selection of 10 tillers per plant for determination of endophyte status. Because of the length of time between harvesting and examination, saprophytic fungi had proliferated in some samples making the endophyte difficult to identify. Results from these contaminated samples (25 plants) were subsequently discarded.

Trial 3

Planter boxes containing the plants from Trial 2 were retained over the winter in the screenhouse. In September 1996 all surviving plants were transplanted individually into 12 cm diam pots containing a 50:50 mix of soil and commercial potting medium. Adult ASW were collected from the field on 17 January, 1997 and six weevils were released onto each pot on 21 January. All pots were covered with fine nylon net which was removed on 30 January when plants were checked *in situ* for eggs. All plants were harvested on 19 March and all tillers were subsequently checked for ASW larval damage, with 10 tillers per plant also checked for endophyte presence.

All Trials

Plants in all trials were maintained with regular watering and trimming.

Endophyte infection was determined by staining an epidermal strip from the leaf sheath of each tiller in aniline blue and microscopic examination at 200x magnification. All samples were checked twice independently of previous results. The identity of the *Gliocladium*-like endophyte in Trial 1 was confirmed by Mike Christensen, AgResearch Grasslands, Palmerston Nth. In Trial 3 samples containing contaminant fungi were checked for the presence of the *Gliocladium* endophyte by Dr Margaret di Menna, Ruakura, Hamilton.

Data were analysed by ANOVA without log transformation.

RESULTS

In Trial 1 Argentine stem weevil adult feeding score was significantly higher ($P < 0.001$) on endophyte-free plants (N-Plants), plants with the *Gliocladium*-like endophyte (G-Plants) and on *N. lolii*-infected plants with some endophyte-free tillers (P/N-Plants) than on plants completely infected with *N. lolii* (P-Plants) (Table 1). In Trial 2, P/N-Plants had similar adult feeding scores to P-plants and both of these had lower scores ($P < 0.001$) than the N-Plants. No G-Plants were identified in Trials 2 or 3. In Trials 2 and 3, egg numbers did not differ significantly between any of the plants.

In Trial 1, the 28% of tillers damaged by larvae on P/N-Plants and 29% on N-Plants was higher ($P < 0.01$) than the 12% of tillers damaged on P-Plants, and lower ($P < 0.05$) than the 41% on G-Plants (Table 1). Multiple infections of *N. lolii* and the *Gliocladium*-like endophyte also occurred but larval damage to these plants was the same as to plants infected with *N. lolii* only. Hence data for these two categories were combined for the P-Plants.

In Trials 2 and 3, P-Plants had the lowest levels of larval damage, N-Plants the highest and P/N-Plants were intermediate between these. These differences were significant ($P < 0.001$) in Trial 2 but not in Trial 3 when damage levels were generally low and large numbers of plants had no damage.

There were relatively few P/N-Plants by comparison with P-Plants in Trials 1 and 3, but in Trial 2 the number of plants in each of these categories was similar (Table 2). Of the 13 P/N-Plants in Trial 3, six had been P/N-Plants and five had been P-Plants the previous year in Trial 2, while 76% of the P/N-Plants in Trial 2 were found to be fully infected in Trial 3. Many of the N-Plants from Trial 2 did not survive until the following year; hence the fewer plants in this category in Trial 3. The mean percentage of tillers not containing *N. lolii* on P/N-Plants was 21%, 30% and 19% in Trials 1, 2 and 3 respectively (range for individual plants was 1 - 80%).

TABLE 1: Mean Argentine stem weevil adult feeding score (FS), number of eggs and proportion of tillers with larval damage on Grasslands Greenstone ryegrass with variable endophyte status.

	Endophyte status ¹			
	P-Plants	P/N-Plants	N-Plants	G-Plants
Trial 1				
FS/tiller	0.26 (0.06) ²	1.04 (0.27)	0.93 (0.22)	1.03 (0.13)
Propn. tillers with larval damage	0.12 (0.04)	0.28 (0.05)	0.29 (0.08)	0.41 (0.09)
Trial 2				
FS/plant	1.77 (0.17)	1.61 (0.11)	2.57 (0.25)	
No. eggs/10 tillers	0.45 (0.19)	0.58 (0.19)	0.78 (0.35)	
Propn. tillers with larval damage	0.03 (0.008)	0.11 (0.01)	0.24 (0.02)	
Trial 3				
No. eggs/plant	0.62 (0.19)	1.00 (0.42)	0.67 (0.31)	
Propn. tillers with larval damage	0.03 (0.006)	0.08 (0.03)	0.13 (0.06)	

¹ P-Plants = all tillers on a plant infected with *Neotyphodium lolii*; P/N-Plants = plants infected with *N. lolii* with some endophyte-free tillers; N-Plants = no endophyte present; G-Plants = plants infected with *Gliocladium*-like endophyte

² SEM

TABLE 2: Total number of plants checked in each trial and the percentage of those plants in each endophyte category (see Table 1).

Trial No.	No. of plants checked	Endophyte category ¹			
		P-Plants	P/N-Plants	N-Plants	G-Plants
1	100	76	8	10	6
2	87 ²	36	38	26	
3	86	71	15	14	

¹ See Table 1

² Excludes 25 plants in which fungal contamination of samples made identification of endophyte difficult.

DISCUSSION

Adult ASW are highly selective in their feeding and oviposition habits and this has been demonstrated in a ryegrass pasture dominated by *N. lolii*-infected plants when the *N. lolii*-free plants were sought out and preferentially attacked by this pest (Popay and Mainland 1991). This discriminatory behaviour may therefore at least partly account for the increase in ASW larval damage to partially infected plants shown in the results presented here. ASW adult feeding also increased on these plants in Trial 1 but not in Trial 2 when feeding was recorded in November, 4 months prior to determination of the endophyte status of the plants. Low egg numbers in Trials 2 and 3 meant that no significant differences in oviposition could be demonstrated.

The concentration of the ASW feeding deterrent, peramine, is also critical to maintaining a high degree of resistance to ASW (Popay and Wyatt 1995). Peramine is freely translocated within the above-ground parts of the plant so that it could be expected that endophyte-free tillers on *N. lolii*-infected plants would still contain this alkaloid. The occurrence of such tillers on otherwise infected plants, however, may be indicative of a lack of *N. lolii* vigour and by inference a low endophyte concentration. Since *N. lolii*

concentration has been shown to be related to peramine concentration (Ballet *et al.* 1995) it is possible that reduced levels of peramine may also explain the increase in ASW damage to partially infected plants.

Partial infection of plants appears to be a variable occurrence, with numbers of plants recorded with this endophyte status in Trial 2 much higher than was recorded in the other two trials. Plants in Trial 2 also had higher proportions of their tillers recorded as *N. lolii*-free compared with those in Trials 1 and 3. Furthermore the majority of the plants found to be partially infected in Trial 2 were found to be fully infected in Trial 3. These results suggest environmental, rather than genetic, factors may be responsible for this variable endophyte status. Infected plants recorded with *N. lolii*-free tillers in Trial 2 had been kept in planter boxes for 7 months before harvesting by which time they were severely root bound and had also suffered a severe outbreak of rust disease. These circumstances may have contributed to the high incidence of *N. lolii*-free tillers.

Occurrence of the *Gliocladium*-like endophyte in Trial 1 was associated with an increase in larval damage to these plants. These results are in line with those of Gaynor *et al.* (1983) who showed preferential feeding by adults on leaf blades from ryegrass plants infected with this fungus. The results presented in this paper also confirm the finding by Gaynoret *et al.* (1983) that the effect of the *N. lolii* endophyte is dominant where co-infection with the *Gliocladium*-like endophyte occurs.

It is not known what the incidence of endophyte-free tillers on *N. lolii*-infected plants is in the field but it seems unlikely to be high based on the results presented in this paper. Given also the relatively low numbers of endophyte-free plants and the infrequency of infection by the *Gliocladium*-like endophyte, it seems unlikely that the variable infection status of Grasslands Greenstone places this ryegrass at risk of severe damage by ASW in the field.

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