ENDOPHYTES IN NORTHLAND ECOTYPES OF HYBRID RYEGRASS

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SUMMARY

Ryegrass (*Lolium* spp.) ecotypes collected in Northland which resemble Italian ryegrass (*L. multiflorum*) contained *Acremonium lolii*, as well as the *Acremonium*-like and *Gliocladium*-like endophytes which have previously been shown to infect Italian ryegrass. *Acremonium lolii* was stably inherited in these Italian-like ecotypes. Leaves from plants containing *Acremonium lolii* deterred Argentine stem weevil (*Listronotus bonariensis*) feeding, but leaves from plants containing the other endophytes did not.

INTRODUCTION

Italian ryegrass (*Lolium multiflorum*) is widely presumed to be susceptible to Argentine stem weevil (ASW; *Listronotus bonariensis*) attack. The Italian ryegrass cultivars and similar unregistered seedlines available to farmers in New Zealand do not contain *Acremonium lolii*, the endophytic fungus which provides some perennial ryegrasses with resistance to ASW (Forde *et al* 1988; Latch *et al* 1988). A recent collection of ryegrass in 'old' permanent pastures in Northland (unpublished data) exposed four sites where all plants collected were Italian in character. Fungal endophyte screening indicated that a variety of endophytes were present. This paper reports the types of endophyte found, their inheritance, and the deterrence of ASW feeding by these ryegrasses.

METHOD

From a sampling of ryegrass plants at 38 sites across the Northland peninsula in January 1986, four sites provided Italian-like types only and no perennial ryegrasses; i.e. the plants had root exudates which fluoresced, poor summer growth, and awned seeds. The sites were near Te Hana, Titoki, Waimamaku, and Awanui. Parents (i.e. tiller propagated descendents from the original collection) and seed-propagated offspring were compared in a randomised block, spaced-plant trial in 18 replicates with four plants per plot, planted in April 1987 at the Kamo Agricultural Centre. This trial included parents and offspring from other Northland sites and exotic seedlines of Italian and perennial ryegrasses as comparative controls.

Between December 1987 and February 1988, three leaf sheaths were removed from one plant per plot of each of the four ecotypes. The sheaths were inspected for fungal endophyte mycelium after staining with lactophenol-aniline blue and mounting in lactophenol.

Plants with or without the various endophytes, being either ecotypes or exotic cultivars of Italian or perennial types, were removed from the Kamo field trial in February 1988 and grown on in a shadehouse. Leaves (one from each plant per replicate) were sellotaped in random order onto a 'comb' (Kain *et al* 1982) and placed with moistened filter paper into a translucent plastic container with 20 field-collected ASW adults. Each comparison container was replicated 13 times and held in a controlled environment (18°C and 12:12 L:D). Leaves were scored daily for 5 days for ASW feeding on a 1-5 scale.

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RESULTS

Three types of fungal mycelium were found. These appeared similar to the *Acremonium lolii* of Latch *et al* (1984), the *Gliocladium*-like species of Latch *et al* (1984), and the *Acremonium*-like species of Latch *et al* (1988). We did not isolate or culture each type as proof, but the identifications were confirmed by G.C.M. Latch (pers comm.).

Of the 144 plants examined for endophytes, two did not produce an adequate leaf sheath sample. Of the balance, 119 (or 84%) had at least one of the endophyte types. *Acremonium lolii* was present in 82 plants, but there were differences between sites (Table 1). The other two endophytes have been grouped together in Table 1, because of uncertainty in distinguishing between them. It was assumed that *A. lolii* and the *Acremonium*-like species did not occur in the same plant based on light-microscope methodology; where two types occurred we have assumed that one was the *Gliocladium*-like type.

TABLE 1: Presence of endophytes in plants from each site.

	Te Hana	Titoki	Site Waimamaku	Awanui
Numbers of Plants:				
Without endophyte	1	9	4	11
With endophyte	35	27	32	25
% With <i>Acremonium</i> <i>lolii</i> only	9	7	78	20
% With <i>A. lolii</i> + Other	80	41	22	64
% With Other only	11	52	0	16

TABLE 2: Feeding score, at day four, for ASW damage to leaves of various endophyte containing plants of Italian and perennial ryegrasses.

	Feeding score
No Endophyte	
“Ruanui” (perennial)	2.4
“Paroa” (Italian)	2.8
<i>Acremonium lolii</i>	
“Ellett” (perennial)	1.2
Local perennial ex Ruawai	1.1
Te Hana	1.2
Titoki	1.2
Awanui	1.2
<i>A. lolii</i> + <i>Gliocladium</i> -like	
Te Hana	1.0
<i>Acremonium</i> -like	
“Concord” (Italian)	3.1
Awanui	2.7
LSD 5%	0.6

With parent-offspring comparisons, 52 of a total of 70 pairs of plants examined had endophytes in both parent and offspring. *Acremonium lolii* was present in the offspring of 92% of parents with this type, which, considering errors in identification,

suggests that *A. lolii* was stably inherited with these ecotypes.

The results of ASW feeding were clear cut (Table 2); there was no evidence that the presence of any endophyte other than *A. lolii* deterred ASW feeding.

DISCUSSION

The principal finding of this work is that *Acremonium lolii* is present in Italian-like ecotypes. Work is needed to define the taxonomy of these ecotypes, but at this stage they are thought to be Italian-perennial hybrids based on the presence of such perennial ryegrass characters as narrow leaves and fineness of the tillers. There was a regression to perennial ryegrass characters in the progeny of controlled crosses (unpublished. data). If they are hybrids then they probably gained *Acremonium lolii* as perennials prior to "natural" selection towards the Italian type.

The demonstration from the parent/offspring trial that *Acremonium lolii* is inherited in these Italian-like ecotypes is the second important finding. Since Italian ryegrass can now be artificially infected with *Acremonium lolii* (G.C.M. Latch, pers. comm.) this finding has little commercial value in itself, except that it does give plant breeders extra confidence that stable *Acremonium lolii* infections may be maintained in Italian ryegrass. The ability to provide Italian ryegrass with "protection" against ASW would be of considerable value to New Zealand's pastoral farming. Also of some practical value is our finding that *Acremonium lolii* was the only endophyte to deter ASW feeding. This simplifies the maintenance of ASW-deterrent ryegrass cultivars and breeding lines. Whether the other endophytes deter other pests is not known.

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