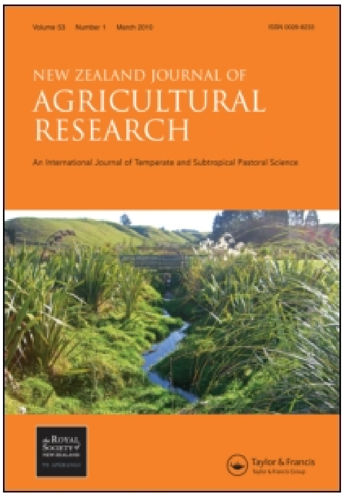

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SHORT COMMUNICATION

Development of a bird-deterrent fungal endophyte in turf tall fescue

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Two *Neotyphodium* fungal endophyte strains (AR601 and AR604) that produce high levels of ergovaline and loline alkaloids were inoculated into turf tall fescue for the purpose of producing symbioses that deter birds. The endophyte–grass associations were shown to be stably transmitted and, in preliminary experiments, reduced insect and bird numbers at an airport and reduced faecal contamination of a sports field by birds. Cultivar 'Jackal' infected with endophyte strain AR601 has been released commercially.

Keywords: AR601 endophyte; ergovaline; loline; *Neotyphodium coenophialum*; *Festuca arundinacea*

Introduction

Avian problems in recreational areas and airfields are commonly associated with birds feeding in these locations. Food sources include insects associated with the turf, turf foliage and seeds of weed species (e.g. *Poa annua*) (Heather & Robertson 1996) that have invaded insect-damaged turf. The *Neotyphodium* endophytic fungi form mutualistic associations with grasses (especially of *Lolium* and *Festuca*) and produce a range of alkaloids that reduce insect pest damage and herbivore feeding, and enhance persistence of the host grass. For example, ergovaline produced by endophytes in both perennial ryegrass (*L. perenne*) and tall fescue (*F. arundinacea* syn *Schedonorus phoenix*; *Lolium arundinaceum*) reduces damage by African black beetle (*Heteronychus arator*) (Ball et al. 1997). Lolines produced by fescue endophytes, such as *N. coenophialum*, are also active on a range of insect pests (Schardl et al. 2007; Popay et al. 2009). To test the hypothesis that endophyte alkaloids may deter bird feeding, seeds of different endophyte–grass associations were fed to Canada geese (*Branta canadensis*) (Pennell & Rolston 2003). Differences in feed preference were recorded, with high ergovaline seed (11–19 ppm) being rejected at the second exposure while feeding on ergovaline-free seed continued to be high. This is a physiological feeding response known as 'post-digestion feedback' (Mason & Reidinger 1983) or 'condition taste aversion' (Nicolaus et al. 1983). While Conover & Messmer (1996) had previously noted reduced feeding on endophyte-infected grass seed, neither the proportion of endophyte infection nor the associated alkaloids were measured.

The objective of the research reported in this paper was to investigate the possibility of developing an endophyte–tall fescue association that was high in bird-deterrent alkaloids.

Development of endophyte–grass associations

Identification and isolation of high ergovaline endophyte

A database of endophyte alkaloid profiles has been developed for seed lines held at the Margot Forde Forage Germplasm Centre,

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AgResearch Grasslands (BA Tapper personal communication). These data were reviewed and two endophyte-infected tall fescue plants expressing high concentrations of ergovaline were identified. These were from seed lines collected in France by GCM Latch in 1991 (83-1 #213, Castellane, Alpes de Haute Provence) and from along roadsides of the Manawatu (New Zealand) (Christensen et al. 1998). The endophytes from these seed lines were isolated using the method described by Christensen et al. (1998) and were named AR601 and AR604, respectively. Samples of these endophytes have been deposited at the National Measurement Institute, Australia. This is an international depository authority in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The deposit numbers are V07/029058 and V07/029061 for AR601 and AR604, respectively.

Endophyte inoculation into cv. 'Jackal'

The continental-type turf tall fescue cultivar 'Jackal' (bred by Keith Saulsbury, Kimihia Research Station, PGG Wrightson Seeds, Lincoln, New Zealand) was used as the host for the two selected endophytes. Sixty seedlings of cultivar 'Jackal', for each endophyte, were infected using the method described by Latch & Christensen (1985). In winter 2005, these seedlings were transplanted into mini seed multiplication field blocks at Lincoln. At seed harvest (end of December), all plants were hand harvested separately.

Re-growth from the spaced plants was sampled in early autumn 2007 (15 March) by cutting to ground level vegetative tillers formed since the seed harvest. The pseudostems were freeze-dried, ground to pass through a 1 mm sieve and analysed for ergovaline and loline alkaloids. Ergovaline was analysed by high-pressure liquid chromatography using procedures based on the work of Spiering et al. (2002), where ergovaline concentrations reported are the sum of measured ergovaline and ergovalinine. Lolines were measured using gas chromatographic methods modified from Kennedy & Bush (1983) and Yates et al. (1990).

For a single germplasm pool there was a wide range in both ergovaline and loline concentrations (Table 1), similar to the 2.5–27.2 ppm range reported by Latch (1994) in ryegrass inoculated with endophyte. While comparisons are difficult with other datasets due to differences in plant part, time of sampling and analytical methods, the mean values for AR601 and AR604 (8.0 ppm) were high (Christensen et al. 1998; Burns et al. 2006). There are three recognised loline fractions produced by *N. coenophialum*: NAL (*N*-acetyl loline), NANL (*N*-acetyl norloline) and NFL (*N*-formyl loline). In AR601 and AR604, 80% or more of the total lolines were contributed by NFL (Table 2).

Endophyte transmission into seed for each plant was confirmed by the seed squash method (Latch et al. 1987) and a bulk from all the seed squash positive lines was then tested for transmission in six-week-old seedlings grown from the seed.

Seed multiplication and endophyte transmission and stability

A bulk seed lot was constituted from plants with both high ergovaline and high loline concentration. This seed was sown in autumn 2006 for a Nucleus 1 seed increase harvested in

Table 1 Ergovaline and total loline alkaloid concentrations in tall fescue pseudostem in early autumn 2007 at Lincoln. Means and range of concentrations for the number of plants tested.

Endophyte	Number of plants	Ergovaline (ppm)		Lolines (ppm)	
		Mean	Range	Mean	Range
AR601	52	8.3	3.4–15.9	1820	1160–3150
AR604	26	7.7	0.0–29.5	1790	540–4060

Table 2 Mean concentrations of loline fractions and contribution to total loline concentrations in tall fescue pseudostem in early autumn 2007 at Lincoln.

	Loline fraction[1]			
Endophyte	NAL	NANL	NFL	Total
AR601				
Concentration (ppm)	150	10	1660	1820
Contribution to total (%)	8	0.6	91	100
AR604				
Concentration (ppm)	110	250	1430	1790
Contribution to total (%)	6	14	80	100

[1]NAL (*N*-acetyl loline), NANL (*N*-acetyl norloline) and NFL (*N*-formyl loline).

summer 2006/07 and re-harvested as a Year 2 crop in summer 2007/08. Seed of the Year 1 harvest was sown in autumn 2007 for Nucleus 2 harvest in 2007/08. The first commercial Breeders seed multiplication was sown in autumn 2008 and harvested in January 2009.

Autumn-sown 'Jackal' AR601 and AR604 produced moderate to high seed yields in the first harvest year (1160–1860 kg/ha) with a high rate of endophyte transmission (94–100%). High transmission rates also occurred for other harvests. Accelerated ageing (40°C at 100% relative humidity) of the seed demonstrated high endophyte stability with 100% viable endophyte after four days ageing and 89% and 90% viable endophyte after five days ageing for AR601 and AR604, respectively.

Insect and plant persistence data

A small-plot randomised block ($n = 2$) experiment was sown in early autumn (16 March) 2006 at Christchurch International Airport (43°29′S; 172°35′E, 40 m a.s.l.) to evaluate AR601 and AR604 in cv. 'Jackal', in comparison with endophyte-free (Nil) 'Jackal' and cv. 'Currawong'. As there was no wild-type endophyte-infected 'Jackal' available to act as a control treatment, 'Currawong' was included as it is a commercially available turf tall fescue infected with wild-type endophyte and produces the endophyte alkaloids of interest in this study. When assessed in autumn 2006 'Currawong' had a lower proportion of endophyte-infected tillers than AR601/AR604 (Table 3). Plots 43 m^2 in size were direct drilled at 200 kg/ha equivalent. Plots were mown when plants reached a height of 150 mm, to a residual height of 40 mm. The site had a low soil fertility, so ammonium sulphate fertiliser (N–P–K–S: 31–0–0–14) was applied at a rate of 125 kg/ha in April 2006.

Insect density was assessed on four occasions from early spring (September 2006) until late summer (February 2007) using a purpose-built vacuum centrifuge at 20 locations in each plot (0.4 m^2 in total) for one replicate. Insects were extracted using a Berlese funnel then oven dried. The major insects collected were coleopteran beetles, weevils (Coleoptera; Curculionidae), collembolan, crane flies (Diptera: Tipulidae) and dipteran flies other than crane flies. Small numbers of thrips (Thysanoptera), leafhoppers (Hemiptera), mites (Acari) and occasionally lepidopteran moths were also caught in samples. The relative insect weight (mean of four sample times) was considerably lower in AR601/AR604 plots than 'Jackal' Nil plots (Table 3).

Tall fescue plant density 1.5 years after sowing was evaluated using a 50-point line transect per plot. The density of plots with endophyte was 2–3-fold greater than the endophyte-free 'Jackal' plots, although this was not statistically different (Table 3). Concentrations of endophyte alkaloids were assessed six times between February and December 2007. When measured on the same dates, concentrations were higher in 'Jackal' AR601/AR604 than 'Currawong' by 3.5–7-fold

Table 3 Proportion of tall fescue tillers infected with endophyte, relative insect weight (mean of four assessments), tall fescue plant density, loline and ergovaline concentrations (total tiller to ground level) at Christchurch International Airport. Statistical analysis was by unbalanced ANOVA.

Cultivar	Endophyte	5 May 06 Endophyte (%)	2006/07 Insect weight	12 Sep 07 Plant density (%)	2007[1] Loline[2] (ppm)	2007[1] Ergovaline[2] (ppm)
'Jackal'	Nil	0	100	33	—[3]	—[3]
'Jackal'	AR601	90	18	87	1410	1.2
'Jackal'	AR604	95	21	69	690	1.0
'Currawong'	Wild-type	75	—[3]	59	200	0.5
	Significance, *P*	0.039[4]	0.082	0.262	0.010	0.480
	LSD 5%	28	61	32	560	1.3

[1] Mean of two dates (February and October) when all treatments were measured at the same time.
[2] Concentrations adjusted to equivalent 100% endophyte tiller infection.
[3] Not measured.
[4] Statistical analysis excludes 'Jackal' Nil.

for lolines and 2–2.4-fold for ergovaline, although the latter was not statistically different (Table 3).

Preliminary bird data

Christchurch airport

Plots of 0.25 ha of cv. 'Jackal' AR601 and 'Jackal' AR604 were direct drilled into resident turf killed with glyphosate in an area adjacent to the insect and plant persistence plots described above. Seed was sown at 200 kg/ha in early autumn 2007. Controls were four adjacent plots with the resident plant species of browntop (*Agrostis tenuis*), Chewings fescue (*F. rubra* ssp. *commutata*), cocksfoot (*Dactylis glomerata*), twitch (*Elytrigia repens*), Yorkshire fog (*Holcus lanatus*), sweet vernal (*Anthoxanthum odoratum*), narrow leaf plantain (*Plantago lanceolata*) and yarrow (*Achillea millefolium*); all are typically found growing in low-fertility soils in this region. Of the grasses, only the Chewings fescue has been reported to host a fungal endophyte (Saha et al. 1987; Rolston et al. 2002). Birds, predominately starling (*Sturnus vulgaris*) and goldfinch (*Carduelis carduelis*), were counted at 09:00 and 17:00 hours daily from October 2007 to October 2008. A generalised linear model was used to analyse the total bird counts in a month. Counts were similar for AR601 and AR604 ($P = 0.127$), so data for these two plots were pooled. Over the 13 months, bird counts on AR601/604 plots (mean of 14 per month) were approximately half those on control plots (mean of 33 per month) ($P = 0.043$, chi-square test).

Recreation Park

In early autumn of 2008, a small-plot randomised block ($n = 2$) experiment was established at a recreation/sports field (Donald Park, Christchurch) that was frequently grazed by Canada geese and other herbivorous birds (e.g. paradise shelducks (*Tadorna variegata*)). The plots, 72 m^2 each, were established by direct drilling after the resident turf was killed with glyphosate. Resident grasses, mainly browntop and *Vulpia* hair grass, were also retained in non-sprayed plots. The area was then maintained by Christchurch City Council. Treatments were 'Jackal' AR601, cv. 'Rebel IV' (tall fescue infected with a wild-type endophyte), two endophyte selections in cv. 'Colosseum' perennial ryegrass and resident grasses. Plots were assessed for faecal contamination by counting the number of faecal deposits in mid-autumn (22 May) 2009. All sown areas had significantly less ($P < 0.001$, analysed by analysis of variance (ANOVA)) faecal counts (0.4/m^2) than the resident grasses (3.0/m^2), while the four sown grasses were not significantly different from each other ($P = 0.897$).

Conclusion

'Jackal' AR601 and 'Jackal' AR604 represent the first high-alkaloid endophyte cultivars developed to produce an endophyte–grass association for application as an avian-deterrent turf. AR601 has shown small advantages over AR604 in some experiments, and a decision was made to commercially release AR601 for autumn turf sowings in 2009. These avian-deterrent endophyte–grass associations are the subject of international patent applications claiming priority date 27 April 2007 (Publication Number: US2008299144, WO2008133533, UY31054). The potential for high-ergovaline alkaloid grasses to escape into livestock farming is recognised based on the toxicity of roadside tall fescue in New Zealand (Easton et al. 1994; Christensen et al. 1998). To avoid escapes, a husbandry package has been developed for seed growers, those involved in seed transport and seed cleaning, and also for aviation and recreational turf managers. Further research to identify bird-deterrent alkaloid dose responses would be of value for the future development of endophyte–grass associations for deterring birds.

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References

Ball OJ-P, Miles CO, Prestidge RA 1997. Ergopeptine alkaloids and *Neotyphodium lolii*-mediated resistance in perennial ryegrass against adult *Heteronychus arator* (Coleoptera: Scarabaeidae). Journal of Economic Entomology 90: 1382–1391.

Burns JC, Fisher DS, Rottinghaus GE 2006. Grazing influences on mass, nutritive value, and persistence of stockpiled Jesup tall fescue without and with novel and wild-type fungal endophytes. Crop Science 46: 1898–1912.

Christensen MJ, Easton HS, Simpson WR, Tapper BA 1998. Occurrence of the fungal endophyte *Neotyphodium coenophialum* in leaf blades of tall fescue and implications for stock health. New Zealand Journal of Agricultural Research 41: 595–602.

Conover MR, Messmer TA 1996. Feeding preferences and changes in mass of Canada geese grazing endophyte infected tall fescue. The Condor 98: 859–862.

Easton HS, Lee CK, Fitzgerald RD 1994. Tall fescue in Australia and New Zealand. New Zealand Journal of Agricultural Research 37: 405–417.

Heather B, Robertson H 1996. The field guide to the birds of New Zealand. Auckland, Viking.

Kennedy CW, Bush LP 1983. Effect of environmental and management factors on the accumulation of N-acetyl and N-formyl loline alkaloids in tall fescue. Crop Science 23: 547–552.

Latch GCM 1994. Influence of *Acremonium* endophytes on perennial grass improvement. New Zealand Journal of Agricultural Research 37: 311–318.

Latch GCM, Christensen MJ 1985. Artificial infection of grasses with endophytes. Annals of Applied Biology 107: 17–24.

Latch GCM, Potter LR, Tyler BF 1987. Incidence of endophytes in seeds from collections of *Lolium* and *Festuca* species. Annals of Applied Biology 111: 59–64.

Mason JR, Reidinger RFJ 1983. Importance of color for methiocarb induced food aversions in red-winged blackbirds *Agelaius-Phoeniceus*. Journal of Wildlife Management 47: 383–393.

Nicolaus LK, Cassel JF, Carlson RB, Gustavson CR 1983. Taste-aversion conditioning of crows to control predation on eggs. Science 220: 212–214.

Pennell CGL, Rolston MP 2003. Effect of grass-endophyte associations on feeding of Canada geese (*Branta canadensis*). Proceedings of the New Zealand Grassland Association 65: 239–244.

Popay AJ, Tapper BA, Podmore C 2009. Endophyte-infected meadow fescue and loline alkaloids affect Argentine stem weevil larvae. New Zealand Plant Protection 62: 19–27.

Rolston MP, Stewart AV, Latch GCM, Hume DE 2002. Endophytes in New Zealand grass seeds: occurrence and implications for conservation of grass species. New Zealand Journal of Botany 40: 365–372.

Saha DC, Johnson-Cicalese JM, Halisky PM, Heemstra MI van, Funk CR 1987. Occurrence and significance of endophytic fungi in the fine fescues. Plant Disease 71: 1021–1024.

Schardl CL, Grossman RB, Nagabhyru P, Faulkner JR, Mallik UP 2007. Loline alkaloids: Currencies of mutualism. Phytochemistry 68: 980–996.

Spiering MJ, Davies E, Tapper BA, Schmid J, Lane GA 2002. Simplified extraction of ergovaline and peramine for analysis of tissue distribution in endophyte-infected grass tillers. Journal of Agricultural and Food Chemistry 50: 5856–5862.

Yates SG, Petroski RJ, Powell RG 1990. Analysis of loline alkaloids in endophyte-infected tall fescue by capillary gas chromatography. Journal of Agricultural and Food Chemistry 38: 182–185.