CULTIVAR AND ENDOPHYTE EFFECTS ON A ROOT APHID, *APLONEURA LENTISCI*, IN PERENNIAL RYEGRASS

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ABSTRACT

In a pot trial, fungal endophytes (*Neotyphodium* spp.) with different alkaloid profiles were investigated for their effects on numbers of a root aphid (*Aploneura lentisci*) in perennial ryegrass. Some endophytes were also tested in different cultivars (Nui, Samson and Impact). Two endophytes, AR37 and AR6, strongly suppressed root aphid numbers. Wild-type had fewer aphids than endophyte-free plants and AR1. Endophytes producing peramine only (AR1, AR12, AR22) and an endophyte producing peramine and lolitrem B (AR23) did not differ from endophyte-free. There were no cultivar by endophyte interactions but cultivar affected the strength of aphid response to Wild-type. Comparisons of alkaloid profiles suggest that ergovaline (in Wild-type and AR6) and epoxy-janthitrems (in AR37) may affect root aphids whereas peramine and lolitrem B do not. Differences in herbage dry weight of plants and a significant relationship between herbage dry weight and root aphid numbers indicated this aphid reduces the growth rate of plants.

Keywords: *Neotyphodium*, alkaloids, herbage, roots.

INTRODUCTION

The root aphid, *Aploneura lentisci*, can be found throughout New Zealand on the roots of grasses such as perennial ryegrass (*Lolium perenne*). Cottier (1953) considered this aphid to be of no economic importance but given its ubiquitous nature, the fact that it is present throughout the year and that it is known to reach high numbers in the field, its effects may have been underestimated. In one field trial at Lincoln in Canterbury, the presence of *A. lentisci* together with the pasture mealybug (*Balanococcus poae*) contributed to differences in yield of ryegrasses with and without infection by different strains of *Neotyphodium* fungal endophytes (Pennell et al. 2005).

The fungal endophyte known as AR1 is now widely used in several different perennial ryegrass cultivars throughout New Zealand after its commercial release in 1999. This endophyte only produces the alkaloid peramine while the Wild-type endophyte produces peramine and two mammalian toxins, ergovaline and lolitrem B. In recent years another endophyte, AR37, has been intensively studied in New Zealand and was commercially released to New Zealand farmers in autumn 2007. This endophyte does not produce peramine, lolitrem B or ergovaline, but is known to produce epoxy-janthitrems (Tapper & Lane 2004), although the biological significance of these alkaloids has not yet been determined.

Previous work has shown little difference in root aphid populations between ryegrass infected with the Wild-type endophyte and endophyte-free (Nil) ryegrass (Popay et al. 2004; Pennell et al. 2005). However, ryegrass, cv. Samson infected with AR1, tended to support significantly higher populations of root aphid than either Nil or Wild-type, whereas ryegrass infected with AR37 was almost completely resistant (Popay et al. 2004). In the pot trial reported here, root aphid populations on perennial ryegrass infected with endophytes with a range of alkaloid profiles were determined and comparisons were also

made with some of those endophytes in different cultivars. Herbage and root weights were measured to determine if these varied with root aphid numbers.

METHODS

This trial tested both the effect of different endophytes producing different alkaloids and interactions between cultivar and endophyte on root aphid populations in a pot trial. Three perennial ryegrass cultivars, Grasslands Samson, Grasslands Nui and Grasslands Impact, without endophyte (Nil) or infected with the Wild-type endophyte or AR1, were used. The effect of another endophyte, AR37, was determined in Samson and Nui. Two additional endophytes, AR6 and AR23, which differ in alkaloid content from AR1, Wild-type and AR37 (Table 1) were tested in Nui. Endophytes AR12 and AR22, which have the same alkaloid profiles as AR1, were tested in Samson.

Germinated seed was planted into a potting medium of two parts soil and one part washed river sand in 100 mm diameter plastic pots in August 2002. Twelve replicate pots were prepared for each cultivar/endophyte combination and spare seed was planted into polystyrene trays. Plants were supplied with slow release fertiliser and retained in a shadehouse at Ruakura Research Centre under regular automatic overhead watering.

In November 2002, all plants were checked for the presence of endophyte by tissue print immunoblotting. At the end of December, 10 replicate pots of each treatment with the appropriate endophyte status were arranged in a randomised block design in the shadehouse. Each plant was inoculated with root aphid by removing a piece of infested root from other potted ryegrass plants and inserting this into the soil of each pot.

Three months later pots were destructively harvested. Herbage was cut from each plant at ground level before root aphids were extracted by flotation and wet sieving. Roots from each plant were recovered during the flotation and wet sieving process and were then washed a second time. Both the herbage and roots from each plant were oven-dried at 80ºC and then weighed.

An analysis of variance was carried out on all data with log transformation ($\log_{10}(n+2)$) required to normalise data for the number of root aphid/plant and number/gram of root. Arithmetic means are also presented for the root aphid data. Separate ANOVAs were carried out for balanced comparisons that included cultivar as a factor and for comparisons among different endophytes within each cultivar.

RESULTS

Within the cultivar Nui, the number of root aphid/plant was lower on plants infected with AR6 and AR37 than on all other endophyte treatments ($P<0.001$). Fewer aphids also occurred on plants infected with the Wild-type endophyte than on plants infected with AR23 ($P<0.05$) (Table 1). In Samson ryegrass, AR37 had fewer root aphid/plant than other endophyte treatments and the endophyte-free control ($P<0.001$), while numbers on Wild-type were significantly less than numbers on AR1, AR12, AR22 and Nil ($P<0.05$). Similarly, Wild-type reduced root aphid numbers relative to AR1 and Nil in Impact. Differences were the same for number of root aphid/g of root (aphid loading) (Table 2; data not presented for AR6, AR12, AR22, AR23).

There were no significant endophyte × cultivar interactions. In cultivar comparisons 1 and 2 (Table 2), mean aphid loading was greatest on AR1 and Nil treatments and significantly higher than for Wild-type. In comparison 2, AR37 had a lower aphid loading than other treatments in both Nui and Samson. Mean aphid loading for all endophyte treatments combined did not differ between cultivars.

Herbage dry weight per plant for ryegrass infected with AR37 and Wild-type significantly exceeded that of AR1 and Nil for all cultivars combined and for Samson, but not for Nui (Table 3). In Nui, AR6 had a higher herbage dry weight than AR1 ($P<0.05$). Within Impact, herbage production did not differ between endophyte treatments. A regression using data for AR1, AR37, Wild-type and Nil in the three cultivars found a significant negative relationship between herbage dry weight/plant as the response variable and log number of root aphid/plant (mean herbage dry weight = 2.40 - 0.303 log no. root aphid, $R^2 = 0.421$; $P=0.018$).

TABLE 1: The $log_{10}(n+2)$ (and arithmetic mean) number of root aphid/plant in three ryegrass cultivars containing endophytes with different alkaloid profiles.

	Alkaloid[1]				No./plant		
Endophyte	Pe	Er	Lo	Ja	Nui	Samson	Impact
Nil	–	–	–	–	1.911 (322)	1.925 (244)	2.063 (675)
Wild-type	+	+	+	–	1.854 (207)	1.225 (171)	0.804 (7)
AR1	+	–	–	–	2.171 (363)	2.587 (699)	2.119 (282)
AR6	+	+	–	–	0.631 (9)	nt	nt
AR12	+	–	–	–	nt[2]	2.075 (329)	nt
AR22	+	–	–	–	nt	2.440 (688)	nt
AR23	+	–	+	–	2.561 (750)	nt	nt
AR37	–	–	–	+	0.458 (1)	0.500 (2)	nt
SED					0.3242	0.3514	0.3510

[1]Pe = peramine; Er = ergovaline; Lo = Lolitrem B; Ja = epoxy-janthitrems.
[2]nt – not tested.

TABLE 2: $Log_{10}(n+2)$ (and arithmetic mean) number of root aphid/g of root for comparisons of endophytes in different cultivars.

Endophyte	Nui	Samson	Impact	Mean	SED
Comparison 1					
Nil	1.946 (270)	1.937 (206)	2.091 (903)	1.991 (460)	
Wild-type	1.711 (176)	1.110 (107)	0.685 (4)	1.169 (96)	0.3789[1]
AR1	2.179 (410)	2.462 (657)	2.063 (211)	2.235 (426)	0.2184[2]
Mean	1.945 (285)	1.836 (323)	1.613 (373)		
Comparison 2					
Nil	1.946 (270)	1.937 (206)		1.941 (238)	
Wild-type	1.711 (176)	1.110 (107)		1.410 (142)	
AR1	2.179 (410)	2.462 (657)		2.321 (533)	0.3299[1] 0.1650[2]
AR37	0.484 (2)	0.485 (2)		0.485 (2)	
Mean	1.580 (215)	1.499 (243)			

[1]SED for comparisons of endophyte and cultivar effects
[2]SED for comparisons of means

Wild-type plants had the highest root growth of any of the endophyte treatments, significantly greater than for AR1 and Nil across all cultivars, and greater than AR37 for Nui and Samson data combined ($P<0.05$) (Table 3). Root weight did not differ between endophyte treatments within individual cultivars.

TABLE 3: Herbage and root dry weights (g/plant) for three cultivars of perennial ryegrass infected with different endophytes.

	Herbage (g/plant)			Roots (g/plant)		
Treatment	Nui	Sam	Imp	Nui	Sam	Imp
Nil	1.8	1.7	1.6	1.1	1.1	1.2
Wild-type	1.9	2.3	2.2	1.6	1.7	1.8
AR1	1.4	1.7	2.1	1.0	1.5	1.3
AR6	2.4			1.1		
AR12		2.2			1.7	
AR22		2.1			1.3	
AR23	1.8			1.0		
AR37	1.9	2.5		1.0	1.4	
SED	0.27	0.28	0.31	0.25	0.28	0.32
Means for cultivar groups						
	Nui/Sam/Imp	Nui/Sam		Nui/Sam/Imp	Nui/Sam	
Nil	1.7	1.8		1.2	1.1	
Wild-type	2.1	2.1		1.7	1.6	
AR1	1.8	1.6		1.3	1.2	
AR37		2.2			1.2	
SED	0.16	0.18		0.16	0.17	

DISCUSSION

This study has confirmed previous work showing that AR37 provides its host plant with a high level of resistance to root aphid in the cultivar Samson (Popay et al. 2004) and has demonstrated that this also occurs in the cultivar Nui. Endophyte AR6 provided a level of resistance similar to that of AR37, while the Wild-type endophyte also reduced root aphid numbers. However, AR6 might not continuously provide its host with protection against root aphid since much higher infestations have been observed on other plants with this endophyte (A.J. Popay, unpubl. data). Similarly, effects of Wild-type on root aphid may only be transitory. In a trial sampled on four occasions over 2 years, Wild-type (in Samson) significantly reduced root aphid numbers relative to endophyte-free plants only once (Popay et al. 2004). In the present trial, the effect of Wild-type was particularly evident in Impact whereas in Nui numbers of root aphid were similar to those on Nil. This apparent cultivar effect and the differences observed over time in other trials are probably due to changes in alkaloid levels, or other resistance factors, in roots. Plant genotype, changes in the physiological state of the plant and environmental effects may modify alkaloid expression within the plant (Popay & Bonos 2005).

In contrast to AR6 (peramine and ergovaline), AR23 (peramine and lolitrem B) had very high root aphid numbers (>2000 on one plant). Root aphid numbers on plants infected with AR1, AR12 and AR22 endophytes that produce only peramine, were all similar to those on endophyte-free plants. AR1 plants can have a significantly higher susceptibility to root aphid than Nil plants (Popay et al. 2004; Popay & Easton 2006). In the present trial, although AR1 plants had higher root aphid numbers than Nil in both Nui and Samson, the differences were not statistically significant.

The high variability in root aphid numbers reported previously among different plants within a cultivar (Popay & Easton 2006) was also apparent here for all endophyte treatments (range 0–>1600/plant) except AR37 (0–12/plant) and AR6 (0–72/plant). Such variability seems to be a heritable feature of endophyte-infected plants that have no resistance to root aphid, suggesting that the symbiotic interaction may modify the host plant's nutritional status and thereby aphid performance.

The mechanisms involved in resistance to root aphids are unknown but are likely to be a result of an alkaloid produced by the endophyte. AR37, which is highly resistant to root aphid, produces epoxy-janthitrems (Tapper & Lane 2004) but it is not known if these affect the aphid. Only low levels of epoxy-janthitrems have been recorded in root tissue relative to those in above-ground plant parts (A.J. Popay, unpubl. data). Within Nui, comparisons of the endophytes differing in production of the alkaloids ergovaline and lolitrem B may provide some clues as to which alkaloids may affect root aphid. Peramine was common to all these endophytes and is therefore unlikely to have an influence. High root aphid numbers on plants infected with AR23, which also produces lolitrem B, indicate that this alkaloid is also unlikely to be affecting root aphid. However, ergovaline, which is produced both by the Wild-type and AR6, may be a possible factor in reducing aphid numbers. Like the epoxy-janthitrems, ergovaline has only been recorded at low concentrations in root tissues compared with above-ground plant parts (A.J. Popay, unpubl. data). It is likely that alkaloids are more concentrated in the phloem where the aphids feed since it is the phloem that transports them from the above-ground parts of the plants where the fungus is located.

Aphid feeding on phloem in the roots is likely to lead to roots becoming a carbon sink, directing photo-assimilates away from herbage growth. The differences in herbage dry weight between treatments and the significant relationship between this and root aphid numbers established in this pot trial suggests that the aphids are impairing plant productivity. Plants were not under any other stress, with regular watering and fertiliser applications and no other insect damage was apparent. The high level of resistance to root aphid shown by AR37 may be one of the factors that give plants infected with this endophyte a yield advantage in the field (Hume et al. 2004).

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