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Interactions between host plant genotype and *Neotyphodium* fungal endophytes affects insects

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Abstract. *Neotyphodium* endophytes usually confer resistance on their host grasses to insect attack, but can also occasionally increase host plant susceptibility to particular insects. In either case host plant genotype interaction with the endophyte appears to play a role in determining the strength of the interactions between the insect and its host. An example of this is the relationship between the root aphid, *Aploneura lentisci* and perennial ryegrass infected with different strains of endophyte. Aphid numbers on individual ryegrass plants infected with the Wild-type endophyte are generally similar to, or less than, those on endophyte-free (Nil) ryegrass whereas populations on plants infected with the AR1 endophyte are significantly higher. By analysing for differences between individual plants using data from five different samplings, significant differences in both the total number of root aphid per plant and aphid loading (number/g of root) were found among 20 plants infected with either the Wild-type or AR1 ($P<0.01$). Host plant genotype of Nil plants also significantly affected aphid numbers ($P<0.05$) but not aphid loading. Population growth of aphids subsequently showed a strong effect of plant genotype for AR1-infected plants but not for Nil plants. In a further trial using cloned plants from half-sibling families of both AR1 and Wild-type, there were significant family effects on aphid numbers/plant, suggesting heritable variation for host control and that selections could be made for AR1 plants that supported low numbers of this insect. There was no comparable variation amongst families infected with Wild-type endophyte in the same experiment.

Introduction

Endophytic fungi (*Neotyphodium* spp.) infecting tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*) play an important role in grassland agriculture in New Zealand, Australia and the United States. The symbiosis between these endophytes and their hosts can provide protection against a range of invertebrate pests as well as abiotic stresses such as drought. However, the increases in productivity and persistence of endophytic grasses can be offset by the toxicity that Wild-type endopytes, which naturally infect cultivated ryegrass and tall fescue (*N. lolii* and *N. coenophialum* respectively), cause grazing livestock. In wild populations of tall fescue and ryegrass there is a diverse array of these *Neotyphodium* fungi, some of which do not produce the alkaloids that harm grazing mammals. Although these endophytes retain some of the insect and drought resistance properties of the Wild-type endophytes, they do not always provide robust protection against the same range of pests. The endophyte AR1 (*N. lolii*) introduced into perennial ryegrass cultivars in New Zealand, is one such example. Host plants infected with this endophyte are strongly resistant to the target insect pest, Argentine stem weevil (*Listronotus bonariensis*), which is capable of causing severe damage to grasses in New Zealand. On the other hand, AR1-infected plants also show increased susceptibility to a root aphid pest, *Aploneura lentisci*, compared with endophyte-free and Wild-type-infected plants (Popay *et al*. 2004). Although it is considered to be a minor pest, under stressful environmental conditions

the root aphid may compromise growth and persistence of plants. Research has shown there to be a wide variability in the root aphid populations found on individual plants. Thus research was carried out to determine if this variability was related to plant genotype and was therefore a heritable trait that would enable selection of plants that were less predisposed to root aphid attack.

Methods

Trial 1

In a pot trial, planted in April 2000, root aphid populations were monitored on perennial ryegrass cv. Samson without endophyte (Nil) or infected with the endophytes AR1 or Wild-type. There were 20 plant genotypes for each endophyte treatment, planted as three-tiller ramets into a soil:sand mixture (2:1) in polythene planter bags (PB2) with rows of holes down the sides of the bags. Each of these planter bags was placed inside a larger planter bag with the space between them filled with sand to enable root aphid populations to be monitored on the root outgrowth without destructively harvesting the whole plant. Plants in each of the 20 replicates were arranged in random order on a sand base inside large plastic tubs, with the tubs arranged in two rows of 10. The trial was maintained outside with regular applications of a complete nutrient solution and watering using a hand held hose applied only as necessary.

Root aphids were sampled on the root outgrowth in the sand medium on four occasions (April and September 2001, January and May 2002) over a 2-year period, using a flotation and wet sieving process. The growing medium and roots were washed and the resulting suspension decanted through three sieves (2.00 mm, 710 µm and 210 µm). The two larger sieves were rinsed thoroughly before all material that had collected on the 210 µm sieve was washed into a 70 mL specimen container. Samples were stored at 4°C until counting. The dry weight of root was obtained at each sampling to enable aphid numbers to be analysed as a function of root weight (aphid loading) as well as a total number per plant.

For counting, samples were transferred to a beaker and diluted if necessary to give an amount between 30 and 60 mL. The total amount depended on the size of the original sample and the number of aphids present. The sample was stirred thoroughly to disperse the aphids before a 10 mL subsample was removed to a petri dish base (90 mm diam.) in five 2 mL aliquots, using a pipette. The base of the petri dish was marked with a grid (approximately 1 mm^2) to facilitate counting. Aphids were counted under a stereo microscope at 16x magnification.

Trial 2

To further investigate an apparent plant genotype/endophyte interaction identified in Trial 1, root aphid numbers were counted on 17 half sibling families of a perennial ryegrass breeding population infected with AR1 and three families infected with Wild-type endophyte. Two different plant genotypes representing each half sibling family were sampled from 3-year-old plants growing at Palmerston North. Parts of these plants were then sent to Hamilton in October 2004 where they were each split into three ramets of four tillers. The ramets were planted separately into a 2:1 field soil:sand mix contained in polythene planter bags (PB2). Plants were randomly arranged in 10 rows of four plants within three replicate blocks in a shadehouse with automatic overhead watering. Three weeks later plants were trimmed to a 5 cm residual and reproductive tillers were removed at the base. Thereafter plants were trimmed to 5 cm approximately

every 4 weeks until root aphid population measurements were made in February 2005. Herbage removed at each cut was oven-dried and weighed.

At the beginning of December plants were inoculated with root aphid by placing an infested piece of root near the crown of each plant. Infested roots were obtained from ryegrass and tall fescue plants and each piece was checked for the presence of live root aphid before it was used as inoculum. A few root pieces were checked again 2 days later. One or two live aphids were still present on each piece but no dead aphids were found indicating that the majority of aphids had migrated to the plants. All root pieces were removed after a further 3 days.

In February, 2 months after plants were inoculated with root aphids, root aphid were washed from the roots by wet sieving and counted as described above. A two-tiller ramet was removed from each plant and replanted so that parent plant material could be retained. All remaining root material was washed thoroughly and then oven dried and weighed.

Statistical analysis

All root aphid numbers were log transformed prior to analysis and data were analysed using GenStat. In Trial 1, an analysis of variance (ANOVA) was carried out on aphid numbers on root outgrowth for each plant in the AR1, Nil and Wild-type treatments using the four sampling times as replicates to determine if there were significant differences among the individual plants for each endophyte treatment (i.e. if there was a plant genotype effect). Means were separated using Fishers protected least significant difference test. In Trial 2, ANOVA was again used to compare aphid populations as numbers per plant and per gram of root both at the family level and on plants within families. Plant growth parameters were analysed in the same way but did not require log transformation.

Results

In Trial 1, AR1 plants had significantly more root aphids than either Wild-type or Nil plants (arithmetic means are presented in Table 1). In addition, total root aphid numbers for the four samplings and the mean number/g of root were highly variable on AR1 plants but less so on Wild-type and Nil plants (Table 1). The role of plant genotype in this variability was investigated by analysing for differences between individual plants within AR1, Wild-type and Nil treatments using the population and aphid loading data for each sampling of root outgrowth. There were highly significant differences between plants within the AR1 and Wild-type treatments for both the number of aphids/plant and number/g of root ($P<0.01$) indicating that some of the variability in aphid numbers was linked to plant genotype. The number of aphids on individual plants within the Nil treatments also varied significantly ($P<0.05$) whereas aphid loading did not ($P=0.089$).

Table 1. Descriptive statistics for total root aphid numbers and mean numbers/g of root for perennial ryegrass plants infected with AR1, Wild-type or endophyte-free for four samplings of root outgrowth in Trial 1.

	Total No. root aphids/plant			Mean No. root aphid/g of root		
	AR1	Wild-type	Nil	AR1	Wild-type	Nil
Mean	784	405	491	350	143	156
Median	479	137	203	153	24	105
Standard deviation	884	619	533	577	282	198
Range	8 – 2981	0 - 2039	36 - 1527	2 - 2356	0 -1150	8 - 2026
N[1]	16	20	13	16	20	13

[1] The number of plants which survived for all samplings and on which statistics are based.

There were significant differences among the 17 half-sib families infected with AR1 endophyte in Trial 2 in the numbers of root aphids per plant and the number/g of root ($P<0.001$) (Table 2). Within families, mean populations/plant ranged from 76 to 1821 (overall mean 482) and number/g of root from 120 to 1690. The highest mean population recorded for three replicate plants of a plant genotype was 2413 and the highest number on any individual plant was 5580. Root aphid populations on plants within families did not differ significantly from each other ($P>0.05$). Six families were identified as having low numbers of root aphids per plant (<200) and five of these also had a relatively low aphid loading.

Productivity of plants was also compared among families and plants within families to determine if this was a factor influencing root aphid populations. There was a strong family effect on total herbage and root production, as there was for root aphid numbers, but there were also significant differences between plants within families. Of the five families with the lowest numbers of root aphid per plant, one was amongst those producing the highest amounts of herbage (77), three moderate amounts and two low amounts (Table 2). Taking root production also into consideration three plants were identified which had low root aphid numbers and moderate to good growth (73, 77, 84).

Root aphid numbers/plant and number/g of root on plants within the three families infected with the Wild-type endophyte were all low and showed little variation. Hence there was no significant difference between them.

Table 2. Mean populations of root aphid and yield of perennial ryegrass plants in 17 half sib families infected with AR1 and three with Wild-type.

Endophyte	Family No.	Root Aphids		Plant Yield	
		Log No./plant	Log No./g of root	Shoot DW g/plant	Root DW g/plant
AR1	73	1.003	1.539	2.7	0.8
	84	1.035	1.391	2.9	0.6
	68	1.051	1.649	1.8	0.3
	72	1.193	1.981	2.3	0.5
	77	1.219	1.965	4.0	0.7
	87	1.303	2.145	2.7	0.3
	61	1.446	2.177	2.7	0.6
	70	1.595	2.613	2.7	0.4
	69	1.672	2.613	3.2	0.5
	80	1.718	2.725	2.5	0.5
	78	1.719	2.728	2.9	0.4
	62	1.862	2.564	3.8	0.8
	65	1.948	2.726	3.6	0.9
	71	2.013	3.029	2.2	0.6
	66	2.031	3.053	4.2	0.5
	63	2.313	3.053	4.3	1.0
Wild-type	85	1.020	1.49	2.8	0.6
	74	1.057	1.65	3.0	0.7
	86	1.288	2.03	2.9	0.7
LSD (5%)		0.573	0.721	1.06	0.28

Discussion

Perennial ryegrass infected with the AR1 endophyte is more highly susceptible to root aphid than endophyte-free perennial ryegrass but also shows a high degree of variability in response among individual plants. Other research has also reported enhanced insect performance on endophyte-infected plants where there is no resistance provided by the endophyte (eg. Saikkonen *et al*. 1999, Bultman & Bell 2003). While the mechanisms behind this are not understood, a plant genotype interaction with endophyte appears to play a role. In Trial 1, there was evidence of plant genotype influencing aphid performance in both Wild-type and AR1-infected plants, but this was less pronounced in Nil plants suggesting that a host plant genotype/endophyte interaction may be more important than plant genotype itself in moderating aphid performance. The relatively weak effect of plant genotype alone has been shown in other work comparing root aphid response to ryegrass without endophyte with that of ryegrass infected with AR1 (Popay 2004). In Trial 2 the significant differences in aphid numbers found among half-sib families of ryegrass with AR1 confirm that susceptibility and/or resistance to root aphid is a heritable trait. For Wild-type infected plants in Trial 2, however, there was insufficient variability in root aphid populations among the three Wild-type families to confirm the results in Trial 1.

Plant genotype effects could not be explained by root availability since the aphid loadings generally reflected the numbers/plant. If habitat is not limiting aphid populations then plant chemistry is the most likely basis for the differences observed.

Composition and concentration of amino acids and concentration of sucrose in the phloem are important determinants of aphid performance (Douglas 1993, Karley *et al*. 2002). It is possible that some of these factors are linked to plant genotype/endophyte interactions and may therefore account for the apparent differences in aphid numbers among the AR1 families. We also cannot exclude the possibility of secondary metabolites produced by the fungal endophyte adversely affecting the aphid. Another endophyte, AR37, almost completely suppresses root aphid populations in perennial ryegrass, suggesting the presence of an endophyte-produced compound that adversely affects the aphids (Popay *et al*. 2004).

Plant genotype exerts considerable control over concentration of secondary metabolites produced by the endophyte (Ball *et al*. 1995 a, b; Easton *et al*. 2002). This may be the reason for a high degree of variability associated with inter-plant genotypic differences found in the amount of damage inflicted on AR1-infected plants by black beetle adults (Easton *et al*. 2000). Similarly in tall fescue, a novel endophyte provides some resistance to plant parasitic nematodes (*Pratylenchus* spp.) in one cultivar but not in another (Timper *et al*. 2005). Other aspects of plant growth and mineral uptake have also been shown to vary according to interactive effects of endophyte and host plant genotype (Malinowski and Belesky 1999, Cheplick and Cho 2003, Cheplick 2004).

Plant growth affects the levels of amino-nitrogen in the phloem, which is an important factor contributing to aphid performance (Whitham 1978). Under the high watering and nutrient regime in which the plants were grown, it was not expected that the root aphids would impact on plant growth. Indeed this was not the case since it was apparent in Trial 2 that the highest populations of root aphid and greatest root aphid loadings occurred on plants with high growth rates. The converse, however, did not always hold. Thus, while AR1 plants with poor growth generally had low aphid numbers, three AR1 families were identified that had moderate to high growth rates but low root aphid populations. These plants hold the key to successfully selecting for plants that have a low susceptibility or high resistance to this insect.

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