RYEGRASS ENDOPHYTE INFECTION AFFECTS ARGENTINE STEM WEEVIL ADULT BEHAVIOUR AND SUSCEPTIBILITY TO PARASITISM

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ABSTRACT

Pot experiments were undertaken to investigate whether Argentine stem weevil *Listronotus bonariensis* adults placed on endophyte-infected or endophyte-free perennial ryegrass (*Lolium perenne*) exhibited behaviour differences that may lead to differing susceptibility to attack by the endoparasitoid *Microctonus hyperodae*. There was no difference ($P>0.05$) in the behaviour of weevils on pots of Nui ryegrass infected with either the wildtype endophyte *Neotyphodium lolii* or AR1, a *N. lolii* selection high in peramine but low in other key alkaloids. Although adults were offered no choice, over 60% of the adults were found off endophyte-infected plants during the observation period. Weevils on high endophyte plants were found to spend less time in positions highly favourable for parasitoid oviposition (e.g. feeding $P<0.001$) and more time in unfavourable positions (e.g. crouching $P<0.01$) than found on nil endophyte plants. The presence of parasitoids decreased the level of weevil feeding ($P<0.001$). These differing behavioural responses to endophyte explain an observed inverse linear relationship between peramine and parasitism in the field.

Keywords: *Listronotus bonariensis*, *Microctonus hyperodae*, *Neotyphodium lolii*.

INTRODUCTION

The impact of Argentine stem weevil (ASW) (*Listronotus bonariensis*) on the pastoral industry has been lessened through the successful combination of plant resistance and biological control. Resistance is conferred by the fungal endophyte *Neotyphodium lolii* (Prestidge *et al.* 1982) and biological control using the endoparasitoid *Microctonus hyperodae* (Goldson *et al.* 1994). Research has shown there are interactions between *N. lolii* infected perennial ryegrass (*Lolium perenne*) and *M. hyperodae*. Barker and Addison (1996) demonstrated that *N. lolii*-infected ryegrass retarded *M. hyperodae* larval development in the host compared with uninfected ryegrass. Barker and Addison (1997) subsequently found that although *N. lolii* infection levels did not affect the efficacy of parasitism of naïve *M. hyperodae*, parasitoids with prior experience of hosts feeding on a particular grass had greater success in parasitising hosts on that grass. Recent field studies by Goldson *et al.* (2000) have found a significant tritrophic interaction whereby percent parasitism was inversely related to levels of peramine, the antifeedant alkaloid on which the resistance is based (Rowan and Gaynor 1985). The most likely explanation was that parasitoids were less successful in finding and attacking prey on high endophyte plots (Goldson *et al.* 2000).

This paper reports the results of a study investigating the behaviour of ASW adults placed on endophyte-infected or endophyte-free ryegrass, and whether behavioural differences result in differing susceptibility to parasitoid attack.

METHODS

Plants of perennial ryegrass cv. Nui containing nil endophyte, wild-type *N. lolii* or the AgResearch novel *N. lolii* endophyte AR1, were established in individual pots (95 mm × 80 mm maximum diameter) filled to 20 mm from the top with potting mix. The pots were maintained in a screen house until each had at least five tillers. An epidermal strip of leaf sheath was taken from each plant, stained with aniline blue and examined under a microscope to confirm the presence of endophyte. Before use, test plants were trimmed to 100 mm in height, desiccated sheath material was removed from tillers and the pots were topped with 10 mm of fine light-coloured sand to improve detection of weevils.

Experiment 1 (April 1999)

Experiment 1 was conducted indoors during the evening to optimise both weevil feeding and parasitoid oviposition behaviour. A single plant of each ryegrass line was placed in a large glass beaker so that no leaf tissue was in contact with any surface. ASW adults, from a site near Te Kuiti at which *M. hyperodae* has yet to establish, were starved for 24 h before use. Twenty weevils taken from the pool of starved weevils were placed on each pot. The beakers were covered with cling film to prevent weevil escape and left in the dark for 30 min. Weevil position was thereafter assessed at approximately 15 min intervals over the following 2.5 h. During assessment, only sufficient light was used to enable determination of weevil positions. The weevils were assessed as feeding (positioned head down and abdomen high), crouching, moving on grass, on pot or off pot. When the experiment was completed, the adults were returned to the pool.

This process was carried out on six consecutive evenings. Each batch of three plants was used on two consecutive evenings. Plants were placed in a random order each evening so that position would not influence observations.

Experiment 2 (March 2000)

ASW adults were collected from a site near Invermay at which *Microtonus hyperodae* has yet to establish. *M. hyperodae* adults were supplied from a rearing programme at AgResearch, Lincoln, and were fed a diluted honey solution daily.

Two plants with nil endophyte and two with wild-type *N. lolii* were placed individually in large glass beakers. Ten weevils were introduced onto each plant, and two or three parasitoids into the beaker of one of each pair of plants. This process was repeated on 8 evenings. Weevils exposed to parasitoids were discarded after use. Otherwise, the experimental method and assessments were identical to Trial 1.

Analysis

An analysis of variance was carried out on the data. In both experiments, not all weevils inside the test container were visible at each observation. Therefore the results are expressed as percentages of total weevils visible, rather than total weevils introduced.

RESULTS

Experiment 1

Endophyte-infection caused a marked effect on ASW adult behaviour (Table 1). Whereas 60% of weevils enclosed with an endophyte-free ryegrass plant were observed on the foliage, only around half this number were found on the foliage of AR1 and wild endophyte-infected plants ($P < 0.001$). Of the weevils observed on the foliage, five times more weevils were feeding ($P < 0.001$) and about half as many were crouching ($P < 0.01$) on the endophyte-free plants compared with the endophyte-infected plants. Visual observations suggested that weevils enclosed on the endophyte-infected plants were more mobile. However, although higher numbers were found moving on the foliage, and on and/or off the pot, only the latter observation was statistically significant ($P < 0.05$). No differences in behaviour ($P > 0.05$) were observed between weevils enclosed on plants infected with the wildtype or AR1 endophytes.

TABLE 1: The relative numbers of ASW adults in various positions or exhibiting various behaviours when placed on endophyte-infected (AR1 and E+) and endophyte-free (E-) ryegrass.

Weevil position	Mean % weevils/observation			Significance ¹
	AR1	E+	E-	
Site observed				
on foliage	36	29	60	***
on pot	31	29	17	ns
off pot	33	42	23	*
Activity on foliage				
feeding	11	13	59	***
crouching	53	52	22	**
moving	38	41	26	ns

¹* P<0.05, ** P<0.01, *** P<0.001.

Experiment 2

As in Experiment 1, more weevils (P<0.01) were found on the foliage and feeding (P<0.001) when enclosed with endophyte-free plants, than when placed with endophyte-infected plants (Table 2). Less adults were observed to be actively moving on foliage of endophyte-free plants than on those with endophyte (P<0.05).

Weevil activity was clearly influenced by parasitoid activity (P<0.05). Overall, the presence of parasitoids reduced the percentage of adults found on the foliage (P<0.05), and the level of feeding (P<0.001). Weevils tended to be more mobile (P>0.05) in the presence of parasitoids. The weevil behaviour fluctuated in response to the intensity of searching behaviour during a single evening. For example, at one observation time, when three aggressive parasitoids were active, no weevils could be observed. Often only the weevils in the vicinity of a parasitoid showed evasive rapid walking behaviour. Successful oviposition by parasitoids appeared most prevalent when there was only one parasitoid active in the test container and weevils were settled.

TABLE 2: The relative numbers of ASW adults (% of those observed) in various positions or exhibiting various behaviours on ryegrass with or without endophyte infection and in the presence or absence of *M. hyperodae* adults.

	endophyte			parasitoid			endophyte and parasitoid		
	+	-	Sig. ¹	+	-	Sig. ¹	+	-	Sig. ¹
Site observed									
on foliage	52	66	**	53	64	*	48	73	*
on pot	22	16	ns	21	18	ns	23	14	ns
off pot	26	18	ns	26	18	ns	29	13	ns
Activity on foliage									
feeding	6	33	***	15	24	***	6	42	***
crouching	64	50	ns	55	58	ns	55	45	ns
moving	31	17	*	30	17	ns	39	13	ns

¹* P<0.05, ** P<0.01, *** P<0.001.

DISCUSSION

The results from these experiments confirm both that ASW adult feeding activity is greatly reduced in the presence of *N. lolii* (Prestige *et al.* 1991 and references therein) and that adult motility is increased as suggested by Barker and Addison (1997). The

adult behavioural responses to endophyte are likely to be induced by the same endophyte alkaloids that reduced feeding damage in previous studies, namely peramine (Rowan and Gaynor 1985) and ergotamine (Popay *et al.* 1990). Behaviour of adults was not different on pots of ryegrass infected with the wildtype endophyte or AR1, an endophyte high in peramine but low in other key alkaloids. Therefore it appears that peramine alone is sufficient to induce significant changes in pattern of adult weevil behaviour.

ASW adults are most susceptible to parasitism when they are feeding, and least when they are inactive or moving (Phillips 1995). These experiments showed that around 90% of adults on the infected grasses were in positions unfavourable for parasitoid oviposition. This behavioural response by the weevils is likely to have given rise to the highly significant inverse linear effect of peramine on levels of parasitism found by Goldson *et al.* (2000).

ASW adult feeding activity was reduced in the presence of parasitoids. Observations suggest that when the ratio of parasitoids to weevil adults is high, parasitoids would be less successful in attack, not only through intraspecific competition, but also because of the high level of evasive behaviours induced in the weevil population.

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