EFFECT OF SELECTED *NEOTYPHODIUM LOLII* ISOLATES ON ROOT-KNOT NEMATODE (*MELOIDOGYNE MARYLANDI*) NUMBERS IN PERENNIAL RYEGRASS

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ABSTRACT

Root-knot nematode numbers were assessed in roots of perennial ryegrass plants containing different strains of *Neotyphodium lolii*. The selected endophytes produced mycotoxin profiles differing from those found in most natural associations. Roots of endophyte-free plants contained the highest numbers of nematodes, significantly more than a perennial ryegrass/*N. lolii* association not producing the mammalian toxin, ergovaline. There was also a significant host plant genotype effect on nematode numbers. The results are relevant to the development of grass/endophyte associations non-toxic to livestock but resistant to nematodes.

Keywords: endophyte, *Acremonium*, *Neotyphodium*, alkaloids, *Meloidogyne*

INTRODUCTION

Infection of perennial ryegrass (*Lolium perenne* L.) with the endophytic fungus *Neotyphodium lolii* (Latch, Christensen and Samuels) Glenn, Bacon and Hanlin (formerly *Acremonium lolii*) is associated with grazing livestock disorders such as heat stress and ryegrass staggers (Fletcher 1993), and host grass resistance to some invertebrate pasture pests (Popay and Rowan 1994). These effects have largely been attributed to the presence of mycotoxins. Most natural associations between perennial ryegrass and *N. lolii* in New Zealand produce the mycotoxins peramine, lolitrem B and ergovaline. Peramine is the major feeding deterrent against Argentine stem weevil (*Listronotus bonariensis* (Kuschel)) (Rowan and Gaynor 1986), a serious pest of pastures, whereas lolitrem B and ergovaline are thought to be responsible for ryegrass staggers and heat stress respectively (Gallagher *et al*. 1981; Fletcher 1993), in livestock.

Neotyphodium endophytes are viewed as important biological control agents of invertebrate pests of grasses including plant-parasitic nematodes. *Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon and Hanlin infection of tall fescue (*Festuca arundinacea* Schreb.) has a detrimental effect on several plant-parasitic nematode species including a root-knot nematode, *Meloidogyne marylandi* Jepson and Golden (Kimmons *et al*. 1990). The effect of *N. lolii* infection of perennial ryegrass on nematodes is less well documented. Evidence from New Zealand is conflicting (Yeates and Prestidge 1986; Cook *et al*. 1991; Stewart *et al*. 1993; Eerens *et al*. 1997), and there is no information available on which mycotoxins affect endoparasitic nematodes in perennial ryegrass.

Latch and Christensen (1985) pioneered the production of synthetic grass/endophyte associations as part of a programme to identify combinations which are not toxic to livestock but still confer resistance to insect pests. The objective of our study was to determine if synthetic perennial ryegrass/*N. lolii* associations, which do not produce the livestock toxins lolitrem B and ergovaline, have an effect on a root-knot nematode. *Meloidogyne marylandi*, a nematode found in North American pastures was used, as cultures of New Zealand endoparasitic nematodes of grasses were not available.

METHODS

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Nematode egg extraction

M. marylandi colonies were maintained in a glasshouse in pots containing single bermudagrass (*Cynodon dactylon* (L.) Pers.) plants in sand:soil mix. After washing off most of the soil from the roots with cold tap water, whole root systems of four bermudagrass plants were removed from just below the crown and cut into approximately 3 cm lengths. The root sections were placed in a blender with 200 ml of 1.05% sodium hypochlorite solution and subjected to 60 seconds of blending followed by 5 second bursts of blending each minute for the next 3 minutes. The resulting macerate was passed through a 200-mesh sieve to remove root and soil debris. The nematode eggs were trapped in a second sieve (500-mesh) placed beneath the first. The eggs were thoroughly rinsed in tap water and the total number estimated by counting eggs from a 1 ml aliquot of a 20 ml suspension on a grid using a binocular microscope (x40).

Nematode bioassay

Two weeks before nematode eggs were extracted from the bermudagrass, tillers from 'Grasslands Nui' perennial ryegrass plants containing selected strains of *N. lolii*, as well as tillers from endophyte-free Grasslands Nui perennial ryegrass plants, were removed from mature mother plants and planted individually into Conetainers (Hummert International; 140 deep x 38 mm diameter) filled with a heat sterilised sand:soil mix. The ryegrass/*N. lolii* associations used and their mycotoxin profiles based on analysis of parental lines are shown in Table 1. Eight replicate tillers from four different Grasslands Nui plants of the same endophyte status (genotypes) were used for each treatment (Table 2). The tillers were maintained in the glasshouse and watered as required. After 2 weeks, each Conetainer was inoculated with 1000 *M. marylandi* eggs. Thirty days after inoculation, the experiment was dismantled.

TABLE 1: Mycotoxin profiles, based upon analysis of parental lines, of the perennial ryegrass/*N. lolii* associations.

N. lolii isolate[1]	Peramine	Lolitrem B	Ergovaline and other ergot alkaloids
AR17	+[2]	-	-
AR19	+	+	-
AR20	+	+	-
AR21	+	+	-
AR23	+	+	-
AR24	+	-	-
N. lolii-free	-	-	-

[1]Synthetic ryegrass/*N. lolii* associations provided by G.C.M. Latch, AgResearch, Palmerston North, New Zealand.
[2]Trace quantities detected (<1.0 ppm).

Nematode counts

Tillers were removed from Conetainers and the roots thoroughly washed in cold tap water. Roots were immersed in a 1.5% sodium hypochlorite solution for four minutes with occasional agitation, rinsed with warm tap water on an 80-mesh sieve, and soaked in tap water for 15 minutes to remove residual bleach. The roots were then boiled in a solution of acid Fuchsin for 30 seconds, allowed to cool, and destained in acidified glycerol (Byrd *et al*. 1983). Female *M. marylandi* in the roots were then counted using a binocular microscope (x40), after which the roots were thoroughly washed with warm water, dried at 100°C for five days and weighed.

Statistical analysis

Data were log-transformed and analysed by ANOVA. Actual (untransformed) means and standard errors are presented; the standard errors are based on between genotype variation.

RESULTS

The greatest numbers of nematodes in any of the perennial ryegrass lines were

recorded in *N. lolii*-free plants (Table 2). *M. marylandi* numbers in roots infected with isolate AR19 were the lowest in the perennial ryegrass/*N. lolii* associations tested, and were significantly ($P<0.01$) lower than nematode numbers in *N. lolii*-free grass. Roots of plants infected with isolates AR17, AR20, AR21, AR23 and AR24 supported fewer nematodes than roots of *N. lolii*-free plants, but the differences were not significant. The variation in nematode numbers among different host plant genotypes was significantly ($P < 0.001$) greater than the variation within genotypes.

TABLE 2: Mean numbers of *M. marylandi* females in roots of perennial ryegrass plants infected with selected *N. lolii* isolates, and *N. lolii*-free plants.

N. lolii isolate	Total number of replicates	Number of different genotypes	Mean number of female nematodes /g root	SEM
AR17	8	4	123	65
AR19	8	4	22**	9
AR20	8	4	70	35
AR21	8	4	120	38
AR23	8	4	88	57
AR24	8	4	119	52
N. lolii-free	8	4	210	67

**Significantly different from *N. lolii*-free at $P<0.01$ (*t* test).

DISCUSSION

Resistance against several species of nematode is well documented in natural associations of tall fescue and *N. coenophialum* (Pedersen *et al*. 1988; Kimmons *et al*. 1990). However, the impact of *N. lolii* infection of perennial ryegrass against nematodes is less certain. Cook *et al*. (1991) found no effect of *N. lolii* on *M. naasi* infection, while Stewart *et al*. (1993) found that ryegrass plants infected with *N. lolii* had fewer galls and hosted fewer female *M. naasi* than *N. lolii*-free plants. Also, Eerens *et al*. (1997) concluded that it was unclear whether the lower numbers of *Paratylenchus* observed on *N. lolii*-infected relative to *N. lolii*-free plants was directly caused by the presence of endophyte, as dry matter yields were higher in endophyte-free compared with endophyte-infected plants. Our results do not elucidate these inconsistencies as a different nematode species and different ryegrass/*N. lolii* associations were used. However, the significant reduction in the number of female *M. marylandi* recorded in roots of perennial ryegrass plants infected with isolate AR19 (Table 2) supports the contention that *N. lolii* may impart resistance in perennial ryegrass against certain plant-parasitic nematode species. However, enhanced resistance to *M. marylandi* using *N. lolii* strains producing atypical arrays of mycotoxins in perennial ryegrass was not a consistent phenomenon as five out of the six *N. lolii* strains tested did not significantly reduce nematode numbers. As Stewart *et al*. (1993) and Eerens *et al*. (1997) suggested, differences between endophyte isolates or host genotypes, and the effect that this has on their interaction, are likely to have an impact on expression of nematode resistance. The highly significant effect of plant genotype on nematode numbers in the current study adds credence to this argument and suggests an area where more research is needed.

Resistance to *M. marylandi* was observed in a perennial ryegrass/*N. lolii* association (AR19) (Table 2) not producing the animal toxin ergovaline or any other ergot alkaloid (Table 1) suggesting that ergovaline is not necessary for resistance. This may have significant implications on the development of synthetic grass/endophyte associations for the forage industry, as ergovaline is thought to be responsible for serious health problems in livestock (Fletcher 1993). However, it is not possible to attribute the observed *N. lolii*-mediated resistance against *M. marylandi* to any particular mycotoxin. It is possible that other factors, such as increased chitinase activity (Roberts *et al*. 1992), may be involved as well as, or instead of, mycotoxins.

This study has shown that a novel ryegrass/*N. lolii* association which does not produce the animal toxin ergovaline is resistant to a root-knot nematode. The work shows the need for future studies of synthetic associations of grasses and endophytes to include investigations with nematodes.

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