# Endophyte effects on consumption of seed and germinated seedlings of ryegrass and fescue by grass grub (*Costelytra zealandica*) larvae

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## Abstract

Tall fescue, meadow fescue and ryegrass seeds with and without endophyte infection were fed to third-instar grass grub to determine the relative effects of different endophytes on consumption. Treatments were: tall fescue without endophyte or infected with four novel endophytes (AR514, AR542, AR584, ES), meadow fescue without endophyte or infected with *Neotyphodium uncinatum* and perennial ryegrass infected with a novel endophyte AR37. Grass grub larvae were initially given hard seed but when they failed to eat this, moist soil was added to allow seed to soften and germinate. After 7 days, all endophytes had reduced feeding compared to their endophyte-free counterparts. At the completion of the trial, 15 days after adding moist soil, only AR37 in perennial ryegrass had no effect on damage to the seed. Of the endophytes in tall fescue, seeds containing AR542 were significantly more damaged than other endophyte treatments. Composition of loline alkaloids may be important in reducing feeding.

**Keywords:** tall fescue, meadow fescue, ryegrass, endophytes, loline alkaloids, seed damage, grass grub, *Costelytra zealandica*

## Introduction

Infection of meadow fescue (*Festuca pratensis*) with its natural endophyte, *Neotyphodium uncinatum*, reduces root feeding by grass grub larvae (*Costelytra zealandica*) (Fletcher *et al.* 2000; Popay *et al.* 2003). Similar effects have also been demonstrated in tall fescue (*Festuca arundinacea*) infected with wild-type *N. coenophialum* and some non-toxic endophytes but these effects are inconsistent (Popay *et al.* 1993; Popay unpublished). A similar variability in effects of *N. coenophialum* in tall fescue has been reported for other root feeding scarabs (e.g. Murphy *et al.* 1993; Koppenhoffer *et al*. 2003). There is no conclusive evidence that endophytes in perennial ryegrass have any impact on grass grub although minor effects have been reported in laboratory trials (Prestidge & Ball 1993; Popay *et al*. 2000).

There is considerable diversity of endophytes among grasses. Testing these endophytes in their host plants for their effects on root-feeding scarabs such as grass grub is laborious and time consuming. Measuring damage to seeds, which contain high concentrations of the alkaloids produced by the endophyte, may provide a quick method for identifying those endophytes that are likely to deter these insects. In this paper, the consumption of tall fescue, meadow fescue and perennial ryegrass seed and germinated seedlings without endophyte or infected with different endophytes is compared.

## Methods

Seed treatments used in this trial were: tall fescue cv. Jesup, without endophyte (Nil) or infected with novel endophytes AR514, AR542, AR584 and an experimental strain (ES); meadow fescue (German origin, cultivar unknown) without endophyte or infected with *N. uncinatum*; perennial ryegrass cv. Grasslands Samson without endophyte or infected with the novel endophyte AR37. The alkaloids produced by these endophytes are given in Table 1, along with the percentage of viable endophyte infection previously determined on plants grown from the same seed lines included in this trial using immunoblot procedures. Actual infection rates of seed are likely to have been higher than the viable infection rates in Table 1 because inevitably there is some loss of endophyte viability when seed is in storage. Seeds

**Table 1** Infection rates of viable endophyte in tall fescue, meadow fescue and perennial ryegrass and the alkaloids known to be produced by the different endophytes in the seed fed to grass grub.

| Host | Endophyte treatment | % Viable infection | Alkaloid content[1] |
|---|---|---|---|
| Tall fescue | E- | 0 | |
| | AR514 | 46 | peramine, clavines, NFL, NAL, NANL |
| | AR542 | 100 | peramine, NANL |
| | AR584 | 95 | peramine, NFL, NAL, NANL |
| | ES[2] | 96 | peramine, NFL, NAL, NANL |
| Meadow fescue | E- | 10 | |
| | Wild-type | 71 | NFL, NAL, NANL |
| Ryegrass | E- | 0 | |
| | AR37 | 68 | epoxy-janthitrems |

[1] NFL – N-formyl loline, NAL – N-acetyl loline, NANL – N-acetyl norloline
[2]Experimental strain

**Table 2** Percentage of seed damaged by grass grub larvae after 7 and 15 days in moist soil and the percentage of seeds that were completely destroyed, severely damaged or partially damaged after 15 days.

| Host | Endophyte treatment | % Damaged | % Damaged | % of damaged seeds | | |
|---|---|---|---|---|---|---|
| | | 7 days | 15 days | Destroyed | Severe | Partial |
| Tall fescue | E- | 41 | 100 | 99 | 1 | 0 |
| | AR514 | 17 | 43 | 3 | 21 | 77 |
| | AR542 | 14 | 79 | 49 | 33 | 18 |
| | AR584 | 12 | 60 | 20 | 29 | 51 |
| | ES | 18 | 41 | 21 | 29 | 50 |
| Meadow fescue | E- | 40 | 95 | 68 | 24 | 8 |
| | Wild-type | 13 | 74 | 19 | 20 | 61 |
| Ryegrass | E- | 59 | 96 | 68 | 25 | 7 |
| | AR37 | 26 | 88 | 59 | 31 | 10 |
| LSD (5%) | | 14.72 | 16.27 | 17.95 | 17.90 | 19.07 |

containing endophyte that have lost their viability still retain high alkaloid concentrations.

Third-instar grass grub larvae were collected from the field and were placed individually into 35 mm diam. wells in six-well tissue culture plates on the following day. Twelve larvae were randomly assigned to each treatment and ten seeds of the appropriate treatment were given to each larva. Lids were placed on the tissue culture plates, which were then wrapped in damp paper towels and placed inside sealed plastic bags. The trial was kept at ambient temperatures in the laboratory.

After 5 days, no feeding was apparent on the hard seed. Larvae and seed were removed briefly from each container while a 1-cm layer of moist soil was added to the base of each well. Feeding damage to seed and germinating seedlings was recorded after a further week. Damage to seed ranged from complete destruction with only husks still present to partial or complete severance of the developing seminal roots or coleoptile of the germinating seed. A final assessment of damage was carried out 15 days after moist soil was added to the containers. Seed was classed as partially damaged if either the developing root or shoot had been partly or completely severed but the endosperm remained intact, severely damaged where both root and shoot had been removed and there was surface damage to the endosperm or completely destroyed where the endosperm was partly or completely eaten out. Seed that was missing was considered destroyed.

Data were subjected to analysis of variance in GenStat Version 9. Means were separated using Fisher's protected least significant difference test at $P<0.05$.

## Results

After 1 week in moist soil, endophyte-infected seeds in tall fescue and meadow fescue were significantly less damaged compared to their E- counterparts ($P<0.001$) with no significant differences among the various endophyte-infected treatments (Table 2). Ryegrass seeds infected with AR37 also had less damage than E- ryegrass seeds ($P<0.05$) but damage to AR37 seed was similar to E- tall and meadow fescue and greater than the endophyte-infected seed of these species. Endophyte-free ryegrass seeds were more damaged than E- tall or meadow fescue seeds.

At the final assessment after 15 days, over 95% of the E- seeds of perennial ryegrass, tall fescue and meadow fescue had been damaged (Table 2). All endophyte-infected seed treatments, with the exception of AR37 in ryegrass, significantly reduced the percentage of seed that was damaged. Among the tall fescue endophytes, AR542 had the least effect with significantly more seed in this treatment damaged than seed infected with AR584 ($P<0.05$), AR514 and ES ($P<0.001$). Furthermore, more AR584 seeds were damaged than both AR514 and ES ($P<0.05$).

The severity of damage to seed differed greatly among treatments. The proportion of damaged seed that was completely destroyed was much higher in E- treatments than in wild-type meadow fescue and tall fescue infected with AR514, AR584 and ES (>68% cf. <21%; Table 2). Conversely, over 50% of the damaged seed in these infected treatments suffered only partial damage where only the developing root or shoot was severed. Although AR542 had fewer seed completely destroyed than E- tall fescue, seed destruction was greater in this treatment than in AR514, AR584 and ES. Amongst the tall fescue endophytes, AR514 seed suffered the least damage with only 3% of seed completely destroyed and 77% partially damaged.

## Discussion

Endophytes in both tall fescue and meadow fescue inhibited feeding by grass grub in this trial with considerable variation in the strength of inhibition among different endophytes in tall fescue. AR37 in ryegrass initially appeared to weakly deter grass grub but this effect did not persist and seeds containing this endophyte were severely damaged by the end of the trial.

A previous study demonstrated that crude extracts containing lolines deterred feeding by grass grub larvae (Popay & Lane 2000). This trial provides further evidence that these alkaloids are the likely factors in reducing feeding and, in addition, indicates the importance of particular loline derivatives. In tall fescue the order of response to endophyte in seed from strongest to least was AR514>ES>AR584>AR542. Of these, AR514 seed also contains clavine alkaloids that may have contributed to the strong effect of this endophyte on grass grub. The relative concentrations of NFL, NAL and NANL in tall fescue cv. Jesup seed containing AR542, AR584 and ES (Table 3) suggests, however, that NANL, present in high concentrations in AR542 and AR584 seed, is a

**Table 3** Concentrations (μg/g) of NFL, NAL and NANL (see Table 1) in tall fescue cv. Jesup seed infected with novel endophytes and meadow fescue seed infected with *N. uncinatum*.

| Endophyte | NFL | NAL | NANL |
|---|---|---|---|
| AR514[1] | 1249 | 354 | 0 |
| AR542 | ND[2] | ND | 1767 |
| AR584 | 1156 | 277 | 2091 |
| ES | 2035 | 454 | 147 |
| N. uncinatum | 10659 | 2011 | 1157 |

[1] AR514 levels may be for leaf material
[2] not detectable

weak deterrent to grass grub relative to the high concentration of NFL in ES. Concentrations of NFL in meadow fescue infected with *N. uncinatum* are also high relative to concentrations of NANL (Table 3; see also Justus *et al.* 1997). NFL and NAL have both been shown to inhibit feeding of Japanese beetle (*Popillia japonica*) larvae (Patterson *et al*. 1991) but there is no published information on the effects on insects of these alkaloids relative to NANL.

Meadow fescue seed infected with *N. uncinatum* reduced seed consumption to the same degree as did AR514 and ES in tall fescue which is perhaps surprising given the very high concentrations of NFL occurring in the meadow fescue seed. *In planta* effects of AR514 and ES in tall fescue plants on grass grub, however, have been variable (Popay unpublished) whereas effects of *N. uncinatum* in meadow fescue have been demonstrated in the field as well as in pot trials (Popay *et al.* 2003). Levels of alkaloids in roots are considerably lower than those in the above-ground parts of the plant (Bush *et al.* 1993; Siegel & Bush 1996). Loline alkaloid levels tend to be lower in tall fescue plants infected with *N. coenophialum* than in meadow fescue plants with *N. uncinatum* (e.g. Bush *et al.* 1993; Ball *et al.* 2006) which probably results in a lower expression of alkaloids in the roots which is critical for inhibiting grass grub feeding. Factors in addition to alkaloid concentrations in the plant, such as plant genotype, plant growth and environment, may also influence the internal distribution of the alkaloids in plants.

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