Differential expression of loline alkaloids in perennial ryegrass infected with endophyte isolated from tall fescue

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Abstract

Twenty half-sibling families of perennial ryegrass inoculated with a strain of endophyte isolated from tall fescue were analysed for loline concentrations. Inoculation success varied between families. Frequency of loline expression of different families varied moderately. Family effect on loline concentrations was significant. In another set of material, derived from plants exposed to field selection, endophyte infection frequency in seed harvested from plants verified as infected was close to 100%. Loline concentrations varied significantly between families. For nine of 23 families, none of the three plants sampled showed any measurable loline. As in other data sets, the results show variation in concentrations of endophyte alkaloids controlled by the genetics of the host plant.

Keywords: *Neotyphodium*, host variation, adaptation, symbiosis, lolines

Introduction

Loline alkaloids in tall fescue (*Festuca arundinacea*) and meadow fescue (*F. pratensis*) infected with their *Neotyphodium* symbionts have little or no adverse effects on livestock (Jackson *et al.* 1996), but provide effective protection from invertebrate attack (Bush *et al.* 1997). Infected tall and meadow fescues are resistant to the important NZ pasture scarab beetle *Costyletra zealandica* (Fletcher *et al.* 2000). Perennial ryegrass (PRG, *Lolium perenne*) is not resistant, and expression in ryegrass of loline alkaloids would be desirable. PRG can be infected with *Neotyphodium* isolated from tall and meadow fescue (Christensen 1995).

Herbage of PRG infected with *Neotyphodium* sp isolated from tall fescue contains abundant peramine, higher than found in the tall fescue host plants infected with the same fungal strain (Easton 2006). Fungal secondary metabolism is thus active in a ryegrass host. However, concentrations in the herbage of lolines characteristic of the *Festuca* association are low and variable.

PRG populations infected with strains of *Neotyphodium* from tall fescue known to produce lolines but not ergovaline were analysed for variation in propagation of the symbiont, and production by it of loline alkaloids.

Materials and Methods

Two sets of material were studied.

Breeding pool families inoculated with AR584

Twenty half-sibling progeny families of a breeding pool derived from crossing PRG cultivars Samson and Impact were inoculated with *N. coenophialum* strain AR584 isolated from tall fescue, using an established technique (Latch & Christensen 1985), and infected seedlings grown outside in pots for 18 months. In October (spring) they were harvested close to ground level and roughly dissected into leaf blade and sheath. Leaf sheath samples were snap frozen in liquid nitrogen and stored. Samples were freeze-dried and analysed for lolines by modification of the gas chromatography methods of Kennedy and Bush (1983) and Yates *et al.* (1989).

Families and crosses infected with AR502 and AR525

Seed of PRG cultivar 'Grasslands Samson' free of endophyte was germinated and inoculated with strains AR502 (FaTG-3) and AR525 (*N. coenophialum*), both isolated from tall fescue. Infected plants were pollinated in a large block of Samson plants of varying endophyte status, and seed harvested. Maternal half-sibling families were sown in replicated rows at Kerikeri, Northland, and came under regular summer pressure of drought and insect attack. After 3 years surviving plants from well-performing progenies were recovered. A number were pair crossed with plants from a breeding pool, GA98, and seeds harvested off the Samson parents were grown out and interpollinated. Seed from 23 plants verified as endophyte-infected (four with AR502 and 19 with AR525) was harvested. Seedlings were tested for endophyte-infection by an immuno-blot test of the primary shoot (Wheatley & Simpson 2000), and stood outside in pots for the winter. For each family, three plants were sampled as above in late October and analysed for lolines. From seven families infected with AR525 with promising results, a further set of 60 plants was harvested in November and analysed.

Results and Discussion

Breeding pool families inoculated with AR584

Of breeding pool seedlings challenged with inoculation, over 30% died before testing for infection. Of 268 live seedlings tested, 128 (48%) were infected. This success rate compares well with inoculation of PRG with *N. lolii*, its natural symbiont. Success rate within families varied from 18% to 77% (χ^2 P = 0.04). Genetic variation in the host for success of inoculation has been reported previously (Easton *et al.* 2000).

N-formyl loline (NFL) and N-acetyl norloline (NANL) were detected in 29 of the 86 plants analysed. N-acetyl loline (NAL) was also assayed but not found. This is consistent for results with AR584 in tall fescue. Mean concentrations were 55 $\mu g.g^{-1}$ for NFL and 37 $\mu g.g^{-1}$ for NANL. Total lolines in a plant varied from under 20 $\mu g.g^{-1}$ up to nearly 200 $\mu g.g^{-1}$.

Number of plants tested per family varied from one to 11, and the number with detectable lolines showed some indication of variation (χ^2 P=0.06). Omitting plants with zero values, mean concentration varied between families from 25 to 190 $\mu g.g^{-1}$ (Fig. 1, ANOVA log data P=0.007). One family in particular had 8 of 10 plants tested expressing lolines, with concentrations above 100 $\mu g.g^{-1}$ for six of them.

Families and crosses infected with AR502 and AR525

Over 3 years at Kerikeri, Samson progeny rows infected with tall fescue endophyte persisted and grew as well as Samson controls infected with *N. lolii*. This was a mean effect, with the best progenies consistently out-performing the controls.

The seed harvested from the open-pollinated pair cross plants was nearly 100% infected (465 of 480 seedlings tested). This was not expected. Other results (unpublished) have found seed transmission by PRG of endophyte strains derived from tall fescue to vary but to be well short of 100%. Use of plants

surviving at Kerikeri may have selected for endophyte activity but not for seed transmission.

Twenty-five of 69 plants tested had detectable lolines, and the number per family varied (χ^2 P=0.02). NFL was the only species detected. NAL and NANL were tested for but not found. They are found in tall fescue infected with AR502. Concentrations of NFL varied from less than 30 to more than 400 $\mu g.g^{-1}$. There was no indication of any difference in mean concentrations between plants infected with the respective strains. Families with more than one plant expressing loline had mean concentrations varying from 42 to 315 $\mu g.g^{-1}$. Differences between families were significant (ANOVA log data P=0.041).

Results from the extra 60 plants infected with AR525 were consistent with the above. Thirty-two of the 60 tested had detectable lolines (12 of 21 for the same families at the first sampling), and the number per family varied (χ^2 P=0.02). Concentrations were 80% higher than a month earlier (P=0.06 raw data, NS for logs), and family variation was significant (ANOVA log data P<0.001) and consistent with the earlier harvest (Fig. 2).

All three data sets show strains of tall fescue endophyte effectively established and metabolising within PRG, and efficiently transmitted to seed. These experiments included no tall fescue controls, but loline concentrations were approximately

Figure 1 Herbage concentrations (µg.g-1) of N-formyl loline in ryegrass families infected with AR584 (vertical bars = SE for families with more than one plant sampled).

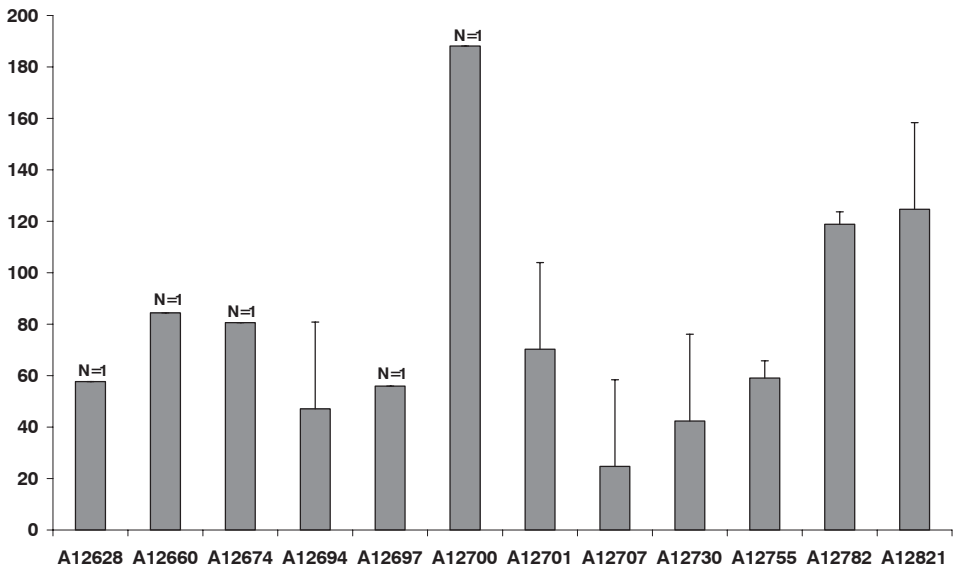
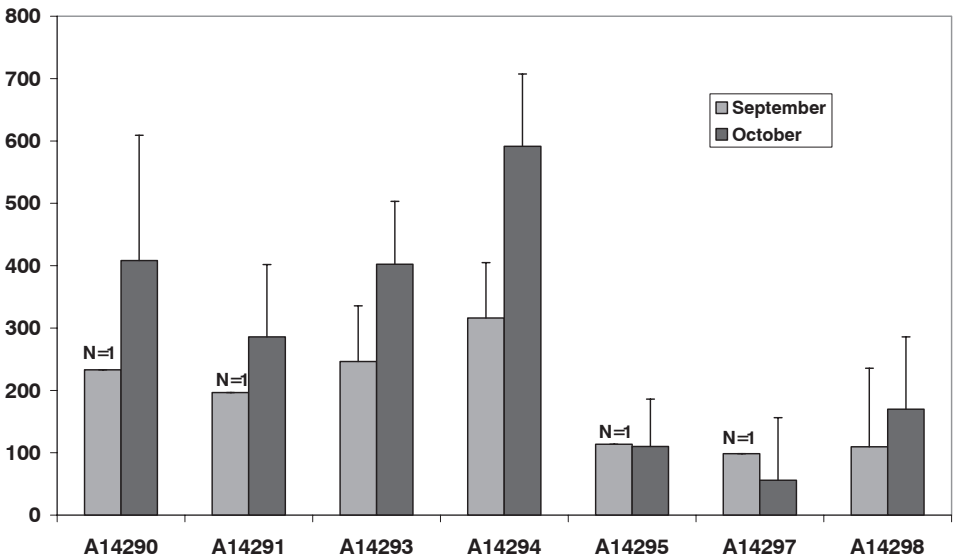


Figure 2 Herbage concentrations (µg.g-1) of N-formyl loline in ryegrass families infected with AR525 (vertical bars = SE for families with more than one plant sampled). Independent sampling in September and October.



one third those expected in spring for tall fescue infected with the same strains. Host family genetic control of alkaloid production by endophyte has been shown for both tall fescue (Hiatt & Hill 1997) and PRG (Easton *et al.* 2002) infected with their respective natural symbionts. These data indicate that continued breeding will develop effective associations between PRG and loline-producing endophyte strains.

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