ROOT LOLINE CONCENTRATION IN ENDOPHYTE-INFECTED MEADOW FESCUE (*FESTUCA PRATENSIS*) IS INCREASED BY GRASS GRUB (*COSTELYTRA ZEALANDICA*) ATTACK

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ABSTRACT

The larvae of New Zealand grass grub are economically important subterranean pests of pastures. Some endophyte-infected meadow fescues contain loline alkaloids in the roots, which can protect the plant from insect attack. Loline concentrations in the roots of meadow fescue ecotypes in autumn were similar to concentrations in shoots of the same line. Loline concentrations in the roots of the meadow fescue ecotypes exposed to grass grub were significantly higher ($P < 0.001$) while concentrations in the crown were lower ($P < 0.05$) than plants of the same ecotypes that were not exposed to grass grub. These results demonstrated that loline alkaloids produced by endophyte-infected meadow fescue can occur in roots at high concentrations (up to $1725 \mu\text{g/g}$), an increase of up to 40% in some lines due to grass grub attack.

Keywords: endophyte, *Costelytra zealandica*, loline alkaloids, pasture, environment, pest, roots, *Festuca pratensis*.

INTRODUCTION

Meadow fescue (*Festuca pratensis*, Poaceae: subfamily Festucoideae) is often infected with the endophyte *Neotyphodium uncinatum*, which has the capacity to synthesise loline alkaloids (Blankenship et al. 2001). Loline alkaloids are potent insecticides (Seigel & Bush 1994) effective on many pasture insects and can act as toxins and feeding deterrents (Dahlman et al. 1991). The concentration of loline alkaloids in roots is generally thought to be much lower than in the crown and shoots of the plant but may be sufficient to protect against some insects (Bush et al. 1997).

Third instar New Zealand grass grub (*Costelytra zealandica* (White) Coleoptera: Scarabaeidae) fed roots of endophyte-infected meadow fescue, in field and laboratory studies, failed to increase their body weight compared to larvae offered meadow fescue roots from plants without the endophyte (Fletcher et al. 2000; Popay et al. 2003). This effect was consistent with the known effects of loline alkaloids extracted from the seed of tall fescue on grass grub larvae (Popay & Lane 2000), although the concentrations of loline alkaloids in the roots of endophyte-infected meadow fescue in the field study were not reported.

This paper reports a field study to determine the effect of grass grub on loline alkaloid concentrations measured in roots, crowns and shoots of 12 endophyte-infected meadow fescue ecotypes. These studies challenge existing knowledge on the relative concentration of loline alkaloids in roots and shoots of fescue and report for the first time an induced increase in endophyte alkaloids directly attributable to insect injury.

MATERIALS AND METHODS

Field experiment

A field experiment was set up at Templeton, Canterbury in February 2005. The site was previously sown with a fine fescue (*Festuca rubra*) and cultivated 6 weeks before transplanting. This experiment was a split-plot randomised complete block design with each plot consisting of two plants of one grass-endophyte combination. In each plot, two x two-tiller ramets from each of 12 grass-endophyte combinations, grown in the same conditions for the previous 15 months, were planted in February 2005 and irrigated to ensure establishment. There were eight replicates of each grass endophyte combination and the control (no plants). One month before the grass grubs were inserted, each plant was separately enclosed by a 20 cm PVC cylinder (internal diameter 16 cm) driven down to ground level. One randomly chosen plant of each pair contained six third instar grass grubs separately inserted 2.5 cm into the soil on 2 April 2005, to achieve a density equivalent to 300 grubs/m². The other subplot was grass grub free. Plants were removed on 11 May 2005, 39 days after insertion of the grubs, complete with the plastic cylinder and enclosed soil. Plants were separated into shoots, crown and roots, weighed fresh, and samples taken for dry matter and loline analysis. The grass grubs were recovered, counted and weighed (data not presented). Herbage samples were pooled from the replicates, freeze dried, ground and kept at -20°C for loline analysis. Loline analyses for each pooled sample were undertaken in duplicate.

Loline analysis

The extraction of loline alkaloids from the plant samples was based on the method of Yates et al. (1990). Dried herbage (0.5 g) was shaken vigorously with 10.0 ml of dichloromethane:methanol:ammonia (75:25:0.5) solvent and 6 mg phenylmorpholine/100 ml of solvent as the internal standard, for 22-24 h. After centrifugation at 2000 g for 15 min, 1 ml of the supernatant was taken up in a 1 ml plastic syringe and passed through a micro-filter (0.45 µm) into a 2 ml glass GC vial for analysis within 24 h.

Extraction and purification of loline dihydrogen chloride, and derivatisation to NFL, NAL, N-acetyl norloline (NANL) and N-methyl loline (NML) were based on the methods of Petroski et al. (1989), Blankenship et al. (2001) and J.D. Blankenship (pers. comm., 2004).

Statistical analysis

The mean loline concentrations in root, crown and shoot for all meadow fescue ecotypes were compared using a paired two-sided T-test (Genstat v.8.2).

RESULTS

Of the grubs introduced into the experimental plots at the start of the experiment 88% were recovered (82% actively feeding, 7% dead).

The total loline concentration measured in the pooled samples could be separated into two widely disparate groups (Table 1); one group of ecotypes (lines Fp53, Fp246 and Fp248) with total loline concentration of 89 µg/g or less and the other lines with 1509 µg/g or greater. At the outset of the research programme all meadow fescue ecotypes were checked for the presence of endophyte by staining with lactophenol cotton blue (Bacon & White 1994). This confirmed the presence of endophyte in all ecotypes except Fp53.

Mean total loline concentration in the roots of meadow fescue plants with grass grubs was higher (26%) than those plants without grass grub ($P < 0.001$, Table 1). Conversely, in plant crowns, mean total loline concentration was 10% lower in plants where grass grubs were present than in plants in plots without grass grub ($P < 0.05$). There was no difference in mean total loline concentration in above ground herbage in the presence or absence of grass grub.

TABLE 1: Total loline¹ concentration ($\mu\text{g/g DM}$) ($\pm$ SD) in the roots, crowns and shoots of 12 meadow fescue lines after 39 days with and without exposure to grass grub feeding in a field experiment.

Line	Root		Crown		Shoots	
	+ grubs	– grubs	+ grubs	– grubs	+ grubs	– grubs
Fp53 ²	89 $\pm$ 57	102 $\pm$ 65	14 $\pm$ 8	38 $\pm$ 9	48 $\pm$ 9	40 $\pm$ 1
Fp246 ²	72 $\pm$ 61	86 $\pm$ 80	10 $\pm$ 1	45 $\pm$ 15	35 $\pm$ 2	47 $\pm$ 8
Fp248 ²	84 $\pm$ 49	123 $\pm$ 75	24 $\pm$ 1	51 $\pm$ 18	30 $\pm$ 4	38 $\pm$ 1
Fp408	1509 $\pm$ 34	1444 $\pm$ 58	1732 $\pm$ 21	1944 $\pm$ 8	1354 $\pm$ 11	1374 $\pm$ 69
Fp87	1556 $\pm$ 4	1334 $\pm$ 126	2415 $\pm$ 7	2766 $\pm$ 0	1751 $\pm$ 62	1513 $\pm$ 31
Fp358	1732 $\pm$ 135	1251 $\pm$ 56	1098 $\pm$ 38	1498 $\pm$ 1	703 $\pm$ 12	907 $\pm$ 49
Fp391	1869 $\pm$ 317	1368 $\pm$ 103	1009 $\pm$ 10	1007 $\pm$ 4	572 $\pm$ 2	772 $\pm$ 8
Fp345	1871 $\pm$ 93	1474 $\pm$ 78	1987 $\pm$ 33	1976 $\pm$ 84	1316 $\pm$ 41	1272 $\pm$ 26
Fp262	1910 $\pm$ 133	1330 $\pm$ 3	1448 $\pm$ 56	1862 $\pm$ 20	1540 $\pm$ 91	1297 $\pm$ 7
Fp440	1920 $\pm$ 19	1725 $\pm$ 18	2377 $\pm$ 271	2281 $\pm$ 4	1374 $\pm$ 13	1528 $\pm$ 281
Fp390	1924 $\pm$ 85	1362 $\pm$ 48	1382 $\pm$ 45	1574 $\pm$ 13	1156 $\pm$ 33	1251 $\pm$ 33
Fp430	1937 $\pm$ 366	1320 $\pm$ 20	1081 $\pm$ 41	1393 $\pm$ 3	751 $\pm$ 11	840 $\pm$ 55
Mean	1803	1401	1614	1811	1169	1195
t-test ³	P<0.001		P<0.05		ns	

¹Total loline concentration = concentration of NFL+NAL+NANL+NML.

²Fp53, Fp246 and Fp248 were excluded from the t-test because of very low loline concentration.

³t-test is a two-sided T-test (paired) Genstat v.8.2.

DISCUSSION

The loline concentration measured in roots of meadow fescue lines in these experiments was high (up to 1937 $\mu\text{g/g}$) compared to previously published data (Bush et al. 1993; Justus 1997) and showed a considerable range depending on specific endophyte-host combinations. The loline concentration in meadow fescue ecotypes in this study that were not exposed to grass grub were on average highest in the crown, lowest in the shoot and intermediate in the roots (Table 1). Previously reported loline alkaloid concentrations in roots of tall fescue and meadow fescue were much lower than the concentrations measured in shoots (Bush et al. 1993; Justus et al. 1997).

In this study, there was an increase in total loline alkaloids in the roots of meadow fescue plants exposed to grass grub larvae and a decrease in the concentration in the crown (Table 1), which may be an induced effect and suggests alkaloid transport to the region of the plant under attack. This finding, if confirmed, does not imply an overall increase in alkaloid production in the plant *per se* but rather a redistribution of lolines in response to an insect attack.

Simulated insect attack by wounding (Bultman & Ganey 1995) and cutting (Bush et al. 1993; Salminen & Grewal 2002; Gonther et al. 2008) increases alkaloid concentration. However, changes in alkaloid concentration caused by wounds inflicted by insect herbivores that are mediated by endophytic fungi have not been documented (Bultman & Ganey 1995). An induced effect was demonstrated when fall armyworm pupae weighed substantially less after feeding on previously clipped, compared to unclipped, endophyte-containing perennial ryegrass (Bultman & Ganey 1995). However, while inducible defense was demonstrated when tall fescue was attacked by fall armyworm, as

measured by an increase in *lola* expression, the same effect was not observed in meadow fescue (Sullivan et al. 2007).

To our knowledge this study is the first demonstration of an induced increase in endophyte alkaloid concentration directly attributable to insect damage. The high (up to 1937 µg/g) concentrations of loline alkaloids found in the roots during autumn-early winter suggest a more important role for loline alkaloids in the protection of plant roots from subterranean insects than previously thought.

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