Response of black beetle and red-headed pasture cockchafer larvae to loline alkaloids in meadow fescue roots

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Abstract Meadow fescue (*Festuca pratensis*) infected with *Neotyphodium uncinatum* endophyte was used to investigate the response of red-headed pasture cockchafer (*Adoryphorus coulonii*) and black beetle (*Heteronychus arator*) larvae to loline alkaloids. Root material of meadow fescue genotypes varying in loline alkaloid concentration were fed to individual larvae in a no-choice bioassay. Total root loline concentrations ranged from 500 to 3000 µg/g DM. Growth of second instar cockchafer larvae was significantly reduced under treatments with highest loline concentrations ($P < 0.05$). Genotypes containing loline concentrations greater than 1000 µg/g DM reduced root consumption of the cockchafer by 11–21% ($P < 0.01$).

Keywords *N*-formyl loline, *N*-acetyl norloline, invertebrate.

INTRODUCTION

Neotyphodium endophytes, which occur naturally in a number of pasture grasses, have been used as an effective tool for control of pasture pests. One of the most common endophytes is *N. lolii*, which occurs in perennial ryegrass (*Lolium perenne* L.) and produces alkaloids in plant shoot material. The alkaloids have been shown to offer protection from Argentine stem weevil (Popay & Wyatt 1995), porina larvae (Jensen & Popay 2004) and adult black beetle (Ball et al. 1994; Popay & Baltus 2001). Trace amounts of *N. lolii* alkaloids (e.g. peramine, lolitrem B) have also been found in root tissue but at concentrations so low (Ball et al. 1997a; Ball et al. 1997b) as to be ineffective in preventing damage by root feeding scarabaeid larvae, such as black beetle (*Heteronychus arator*

[Fabricius]) and red-headed pasture cockchafer (RHPC) (*Adoryphorus coulonii* [Burmeister]).

Popay et al. (2003) showed reduced feeding by grass grub (*Costelytra zealandica* [White]) when offered *N. uncinatum* endophyte-infected *Festuca pratensis* roots compared to uninfected controls and implicated loline alkaloids in this. Loline concentrations within the plant shoot vary (Justus et al. 1997), and while the greatest concentrations occur in the crown and leaf tissue, lolines are also found in the roots (Tong et al. 2006; Patchett et al. 2008). Furthermore, Patchett et al. (2008) showed that under grass grub attack, loline concentrations increased in the roots where defence was required. However, although it has been recognised that increasing

loline content will reduce grub feeding on artificial diets (Patterson et al. 1991; Popay & Lane 2000), the concentration of lolines in root tissue necessary to deter below-ground insects has not been fully investigated.

The objective of this study was to quantify loline concentration in *F. pratensis* and *F. pratensis* × *Lolium* crosses infected with *N. uncinatum* and to examine the effect of variation in loline concentration on larvae of two scarabaeid beetles, black beetle and RHPC.

MATERIALS AND METHODS

Root material

Root material was collected in January 2009 by extracting field-grown, post-anthesis meadow fescue plants (including two hybrid *L. perenne* crosses in their fourth cycle of selection), which had been established in a plant breeding nursery near Darfield in Canterbury (Table 1). Soil was removed from roots, first by hand followed by rinsing with water, before roots were clipped from the plant 2–4 cm beneath the crown. A sub-sample was immediately frozen for later analysis of lolines in freeze-dried material using the method described by Patchett et al. (2008). The *N. uncinatum* strains listed in Table 1 were previously identified as morphologically unique from each other after isolation in culture from host parent material. The presence of endophyte in experimental plants was confirmed following establishment (August 2008) by examination of sheath stained with aniline blue at 400 × magnification.

Insects

Black beetle and RHPC larvae were obtained from established pastures in Northland and Canterbury on 14 and 22 January 2009 respectively. For each treatment, eight each of third instar black beetle and second instar RHPC were weighed into individual plastic ice tray wells. Black beetle larvae were provided with 300 mg of fresh root material and RHPC were given 200 mg fresh root. Trays were then covered and incubated in darkness at 16°C for 7 days. A fasting treatment was also included for each insect group.

On day 7, larvae were removed and weighed, fasted for 24 h and reweighed. The fresh weight of remaining root and any faeces were recorded. The number of larvae that perished during the bioassay was also recorded. Relative differences in liveweight and root consumption between treatments, which were expressed as a percentage of the original weight, were determined by one-way analysis of variance using Genstat (version 11). Correlations between N-formylloline concentration and relative body weight change or root consumption for both larval species were determined by regression analysis.

RESULTS

Although all plants were naturally infected with *N. uncinatum*, two of the seven lines contained no detectable loline in the roots. In the other five lines the concentration of total lolines ranged from 573 to up to 2991 µg/g DM (Table 1). The predominant loline was N-formyl

Table 1 Concentrations of lolines (µg/g DM), N-formyl loline (NFL), N-acetyl loline (NAL) and N-acetyl norloline (NANL), in root material of *F. pratensis* (FP) and *F. pratensis* × *L. perenne* hybrid (FH) lines infected with *N. uncinatum* (U) strains.

Host code	Endophyte		[NFL]	[NAL]	[NANL]
	strain				
FP30	U4		0	0	0
FH146	U8		0	0	0
FP24	U6		573	0	0
FP15	U5		413	56	287
FP39	U10		1380	206	350
FH106	U5		2403	158	188
FP36	U2		2604	176	211

loline (NFL) while N-acetyl loline (NAL) and N-acetyl norloline (NANL) were present in lower concentrations.

The black beetle showed poor resilience to experimental conditions and high mortality (1.75 grubs/treatment) occurred prior to- and during the experiment resulting in exclusion of 22% of data. Larval death was unrelated to loline treatment. Only surviving larvae were used in the analysis and as a result there was large (but not significant) variation in initial liveweight. Black beetle larval mean weight was 406 mg at the start of the bioassay and increased by an average of 6.4%. Although relative weight gain was greatest in the absence of lolines, the differences were not significant. Root consumption was significantly higher in the FP36 association, which had the highest concentration of lolines, than for the other

associations (Table 2). However, in the presence of lolines the dietary response of black beetle larvae was variable so regression analysis did not reveal significant trends with loline concentration.

Mean liveweight of second instar RHPC was 202 mg at the start of the bioassay. The highest weight gains occurred on *Festuca* lines in the absence of loline (Table 2). These larvae also consumed the highest proportion of the roots on offer.

While all RHPC larvae consumed at least 30% of the root on offer and subsequently gained weight, linear regression revealed a significant negative effect of loline concentration on root consumption ($P=0.002$) (Figure 1). The equation predicted a 6% decrease in root consumption for every increase in loline concentration of 1000 $\mu\text{g/g}$ DM.

Table 2 Initial weight, growth and root consumption by black beetle and red-headed pasture cockchafer larvae in a bioassay. Means followed by a different letter within a column are not significantly different according to LSD test ($P<0.05$) following a significant ANOVA.

	Larval weight		Root weight	
	Initial (mg)	Gain (%)	Initial (mg)	Consumed (%)
Black beetle				
FP30	368	8.11	301 b	45.7 b
FH146	411	9.15	302 b	41.1 b
FP24	449	5.25	305 ab	45.9 b
FP15	379	6.13	313 a	43.2 b
FP39	480	3.95	301 b	43.5 b
FH106	358	6.30	294 b	36.0 b
FP36	453	5.96	300 b	66.0 a
Fasted	337	-0.38	-	-
SEM	41.7	2.095	3.6	6.496
Red-headed pasture cockchafer				
FP30	202.9	9.12 a	201.0	43.9 a
FH146	219.1	6.31 ab	200.8	48.6 a
FP24	205.1	5.74 ab	204.2	46.0 a
FP15	178.9	5.02 ab	203.6	39.2 ab
FP39	194.1	2.58 bc	201.6	32.2 bc
FH106	202.6	4.95 ab	203.5	32.8 bc
FP36	219.0	1.20 bc	205.6	27.3 c
Fasted	195.8	-1.59 c	-	-
SEM	21.1	1.863	1.8	3.54

Figure 1 Relationship between total loline concentration in the diet and root consumption by red-headed pasture cockchafer.

DISCUSSION

Root loline concentrations measured in the current study were higher than any documented elsewhere for *Festuca* plants (Potter et al. 1992; Tong et al. 2006; Patchett et al. 2008). The plant material in the present study was similar to that used by Patchett et al. (2008) who recorded root loline concentrations of up to 1900 µg/g DM in late autumn. The higher concentrations reported here are probably due to either the plant/endophyte association or the environmental conditions under which the plant material was collected. In this case, sampling in summer, post flowering (Justus et al. 1997; Tong et al. 2006) and after a long regrowth interval (Kennedy & Bush 1983) are likely to be responsible for promoting the high loline concentrations.

The presence of lolines in *F. pratensis* roots reduced feeding and development of second instar RHPC larvae. Concentrations in excess of 1700 µg/g DM were particularly effective in reducing feeding. Popay et al. (2003) showed a similar response with grass grub feeding on endophyte infected *F. pratensis* although concentrations of loline in roots and subsequent effect on grubs are generally unreported. Loline extracts in artificial diets have been used to demonstrate the negative relationship of loline concentrations on diet consumption by root feeders (Patterson et al. 1991; Popay & Lane 2000). In those studies the diets were homogenous and are unlikely to offer the same

opportunity for diet selection as was available in the combination of young and old root tissue on offer in the present study. Justus et al. (1997) showed large variation in loline accumulation in above-ground plant parts at different ages and stages of reproductive growth, and this is likely to be reflected below-ground in new and existing roots and may subsequently influence feeding behaviour in the rhizosphere.

Although mortality of RHPC was not affected by treatments in this experiment, it is possible the effect of lolines would have been more pronounced over time. Jensen et al. (2009) found increased mortality and reduced feeding of Argentine stem weevil were most evident after 3 weeks on loline-based diets. In contrast, the presence of lolines did not significantly affect black beetle larvae. The lack of responses of black beetle may reflect the more mature larvae used in the study compared with the RHPC larvae. A variable response of older instars was reported by Potter et al. (1992) as their nutritional requirements and metabolism alters over time. Furthermore, there was high mortality of black beetle, due to handling and laboratory conditions, and this restricted replication for robust statistical analysis of the effect of lolines on feeding of black beetle larvae.

The results demonstrate the potential of pasture plants containing lolines for control of RHPC. This pest is known for its ability to cause extensive damage to pasture in Australia (Pavri & Young 2007) as the larvae can reach up to 900 mg in weight at the end of their 2-year lifecycle (Candy & McQuillan 1998). The cockchafer feeds indiscriminately on organic matter in the root zone of the soil hemisphere (McQuillan & Webb 1994). Thus, plant root forms a lower proportion of the diet of this insect compared to other root feeders and even at high loline concentrations the efficacy of the endophyte/plant association may not be sufficient to deter feeding. An example of poor resistance to RHPC was demonstrated by Watson (2007) in pot trials using *N. ceonophialum* endophyte-infected tall fescue, which produces small (relative to *N. uncinatum*) concentrations of lolines in the roots. This needs to be considered in further testing.

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