ENDOPHYTE-INFECTED *FESTUCA PRATENSIS* CONTAINING LOLINE ALKALOIDS DETERS FEEDING BY *LISTRONOTUS BONARIENSIS*

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ABSTRACT

Meadow fescue, *Festuca pratensis*, infected with *Neotyphodium uncinatum* contains loline alkaloids that may deter attack from pasture insects. In a field experiment conducted in Canterbury, high leaf loline alkaloid concentration reduced damage by Argentine stem weevil (ASW; *Listronotus bonariensis*) adults to 12 unique endophyte-infected meadow fescue ecotypes at four assessment times (Dec-Apr; $R^2_{\text{adj}} = 48.9$, $P < 0.001$). In laboratory experiments, ASW adults caused significantly more ($P < 0.05$) feeding damage (number of leaf holes) to nil-endophyte (Fp53) than to endophyte-infected meadow fescue (Fp345). Endophyte-infected *Lolium perenne* (perennial ryegrass 'Bronsyn') sustained least damage.

Keywords: *Listronotus bonariensis*, loline alkaloids, *Festuca pratensis*, endophyte, ecotype.

INTRODUCTION

Meadow fescue, *Festuca pratensis*, is often infected with the endophyte *Neotyphodium uncinatum*, which has the capacity to produce loline alkaloids (Blankenship et al. 2001). Loline alkaloids may deter pasture insects, such as Argentine stem weevil (ASW; *Listronotus bonariensis*), from feeding and egg laying, and may contribute to population control (Popay et al. 2005). ASW is an important pest of pastures in New Zealand and prior to the establishment of effective controls, was estimated to incur costs of \$78-251 million per annum (Prestidge et al. 1991).

Loline concentrations of up to 8000 $\mu\text{g/g}$ have been measured in foliage of tall fescue (Bush et al. 1993). Studies also showed that neither N-formyl loline (NFL) nor N-acetyl loline (NAL) at 100 $\mu\text{g/g}$ were feeding deterrents or toxic to ASW adults (Popay & Latch 1993). However, more recent studies by Miles et al. (1998) and Popay (2004) have implicated both NFL and N-acetyl norloline (NANL) as feeding deterrents of ASW.

The main objective of this study was to determine the effects of meadow fescue ecotypes containing loline alkaloids on feeding by ASW adults in laboratory and field studies. Ecotypes have different loline concentration profiles over time (Patchett 2007), which may influence ASW feeding.

METHODS

Field experiment

This experiment, located near Templeton, Christchurch, comprised a randomised complete block design with three replicates. It was subject to natural infestation by ASW. On four assessment dates (10/12/04, 11/01/05, 3/03/05 and 27/4/05), one or two tillers per plant were taken from 12 meadow fescue lines in each of the three replicated field plots. Feeding holes of adult ASW in all leaves on each tiller were counted at each assessment date. All above ground herbage was cut by hand as close to ground level

as possible and removed from the experimental area immediately after each harvest. Loline concentration was measured in stems and leaves of plants from each plot (ca 0.5 g dried herbage).

Laboratory experiment

A laboratory experiment was undertaken that included the meadow fescue ecotypes Fp53 and Fp345, and the perennial ryegrass 'Bronsyn'. The meadow fescues were selected on the basis of anticipated loline concentration (Fp53 low loline, Fp345 high loline) and 'Bronsyn' because of high peramine concentration, a known ASW feeding deterrent (Rowan 1990).

Rooted tillers were placed in pairs in small polythene pots with a commercial potting mix in a randomised complete block design with five replicates as follows:

Treatment 1: Two separate tillers of Fp53

Treatment 2: Two separate tillers of Fp345

Treatment 3: Two separate tillers of 'Bronsyn'

Treatment 4: One tiller from Fp53 and one tiller from Fp345

Treatment 5: One tiller from Fp53 and one tiller from 'Bronsyn'

Treatment 6: One tiller from Fp345 and one tiller from 'Bronsyn'

The rooted tillers were allowed to establish in the pots outdoors in the autumn of 2004. After 6 weeks three ASW adults, collected from near Hororata, Central Canterbury, and kept in cool storage overnight, were placed with the paired plants. A ventilated clear Perspex cylinder was placed over the plants to contain the weevils and the pots were maintained in an incubator at 20°C and a 16:8 h light:dark cycle.

After 6 days, the weevils were removed and feeding holes in the plants were counted. The plants were then cut at soil level, the tillers and leaves counted, weighed and freeze-dried for loline, ergovaline and peramine alkaloid analysis. The plants comprising each line in each replicate were bulked to give sufficient material (ca 100 mg) for the alkaloid analyses.

Alkaloid analyses

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

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

Peramine and ergovaline analyses were undertaken by AgResearch Ltd by the methods of Rowan et al. (1986) and Davies et al. (1993).

Statistical analyses

In the field experiment the number of ASW feeding holes/plot and concentration of loline alkaloids were subjected to analysis of variance (ANOVA). Regression analysis of ASW damage against the leaf and stem concentration of loline alkaloids was also performed.

In the laboratory experiment the number of feeding holes counted per plant and the loline concentrations in the shoot of each line were analysed using ANOVA to compare feeding levels with each pair of grasses. The number of feeding holes/g and the total number of holes/plant were transformed to meet the equal variance assumptions of the ANOVA.

RESULTS

Field experiment

There were significant differences ($P < 0.001$) in the ASW feeding injury levels (number of holes/plot) between the 12 meadow fescue grass ecotypes at all four assessment times. Generally, at all assessment times, more holes due to ASW attack were counted

in the absence of endophyte than in all other treatments. Ecotypes with high loline concentrations had the lowest number of holes ($P < 0.05$) at all four assessment times (data not shown). More extensive data are available elsewhere (Patchett 2007).

The inverse relationship between the number of ASW feeding holes and total loline concentration (all assessment dates combined) in the plant leaves was significant ($P < 0.05$; $R^2_{\text{adj}} = 13.1$). Fitting separate lines for each assessment date improved the fit of the overall model ($P < 0.001$, $R^2_{\text{adj}} = 48.9$; see equations in Fig. 1). The total loline x assessment time interaction was not significant ($P = 0.077$).

FIGURE 1: Fitted parallel regression lines for the number of ASW feeding holes/plot against total loline concentration ($\mu\text{g/g DM}$) in the leaf of 11 meadow fescue lines assessed four times in 2004/05 ($R^2 = 48.9$, $P < 0.001$). Fp53 was excluded from the regression analysis, as it was a nil endophyte line. Regression equations – Assessment 1: $y = 85.57 - 0.0363$ (total loline); Assessment 2: $y = 100.5 - 0.0363$ (total loline); Assessment 3: $y = 134.73 - 0.0363$ (total loline); Assessment 4: $y = 104.73 - 0.0363$ (total loline).

Laboratory experiment

In treatments with the same grasses no significant difference ($P > 0.05$) was observed in damage by ASW to plants between any of the three grass treatments and there was no difference between treatments in the number of leaves, number of tillers or plant weight.

In treatments with different grasses there were significant differences, after square root transformation, in the total number of holes/plant and number of holes/g between grasses (Table 1). Bronsyn had fewer holes when paired with either of the meadow fescues while Fp345 had fewer holes than Fp53. There was no difference in the number of leaves, number of tillers or plant weight between pairs of plants for any of the comparisons.

The total loline concentration of Fp345 ($7615 \pm 591 \mu\text{g/g}$) was 34–60 times higher than in Fp53 ($222 \pm 111 \mu\text{g/g}$) and 'Bronsyn' ($124 \pm 32 \mu\text{g/g}$). The peramine concentration in 'Bronsyn' ($15.0 \pm 3.0 \mu\text{g/g}$) was higher than in the meadow fescues Fp345 ($3.8 \pm 4.1 \mu\text{g/g}$) and Fp53 ($0.6 \pm 0.7 \mu\text{g/g}$) and had not been detected in these ecotypes previously (Patchett 2007). Ergovaline was found in 'Bronsyn' ($0.38 \pm 0.27 \mu\text{g ergovaline/g}$) but not in Fp53 or Fp345.

TABLE 1: Argentine stem weevil damage (no. holes/g DM) and total no. holes/plant) to two meadow fescue ecotypes and 'Bronsyn' perennial ryegrass in paired comparisons. Values were analysed after logarithmic or square root transformation. A refers to the first grass in each pair, B the second.

Grass choice ¹	A	B	LSD (P<0.05)	P-value
Log(holes/g +1)				
Bronsyn vs Fp345	1.12	3.62	1.60	<0.05
Bronsyn vs Fp53	1.51	3.84	1.41	<0.05
Fp345 vs Fp53	2.33	4.08	2.24	=0.090
SQRT (total holes)				
Bronsyn vs Fp345	1.6	7.6	3.2	<0.01
Bronsyn vs Fp53	2.3	7.7	3.4	<0.05
Fp345 vs Fp53	3.3	8.2	5.0	=0.052

¹Bronsyn=perennial ryegrass; Fp53 and Fp345=meadow fescue.

DISCUSSION

In grasses, loline alkaloids have been associated with the presence of the endophytes *N. uncinatum* and *N. coenophialum* (Blankenship et al. 2001). Differences in damage between ecotypes containing loline alkaloids were observed. Damage was consistently lower in the high loline ecotypes than in the low loline ecotype (Fp53), suggesting a loline effect in reducing leaf damage.

The alkaloids peramine, lolitrem B or ergovaline, which have been shown to deter insect feeding in temperate grasses (Popay & Bonos 2005), were not detected in leaf samples of all the 12 meadow fescue lines collected from the field (11.10.04) (Patchett 2007). Consistent feeding deterrence was shown at leaf loline concentrations greater than 400 µg/g (Fig. 1) (maximum 1476 µg/g), suggesting that the threshold level for feeding deterrence may be considerably greater than 100 µg/g, which Popay & Latch (1993) noted did not deter ASW feeding.

The damage to foliage by adult ASW can provide an indication of oviposition activity and reduced leaf damage may imply that endophyte-produced alkaloids reduce egg laying and larval populations (Popay et al. 2005).

A clear difference in the ASW damage to the perennial ryegrass 'Bronsyn' and the meadow fescues Fp53 and Fp345 was demonstrated in the treatments with different grasses. In the laboratory experiment a concentration of 15 µg/g of peramine, in 'Bronsyn', was sufficient to deter ASW feeding (Rowan et al. 1990). The endophyte-free line Fp53 had very low loline concentration (<50 µg/g) (Patchett 2007), and greater damage to Fp53 than to either Fp345 or 'Bronsyn' by adult ASW feeding was anticipated. The line Fp345 produced high concentrations of loline alkaloids (7615 µg/g) in stems and leaves but 'Bronsyn' was less attractive to ASW for feeding than Fp345. Although other factors, such as high leaf fibre, leaf surface characteristics and internal deposits of silica, may reduce feeding by ASW adults (Barker 1989), these have not been demonstrated in meadow fescue to date.

These results imply that loline alkaloids deter feeding by adult ASW but further studies with purified lolines are required to confirm the toxicity and the feeding deterrence of each loline derivative. Further work is also required to determine the effects of loline-containing grasses on ASW populations in the field.

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