

THE EFFECTS OF LOLITREM B ON ARGENTINE STEM WEEVIL LARVAE

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SUMMARY

In choice tests, Argentine stem weevil larvae were deterred from feeding on artificial diet containing the mycotoxin lolitrem B at 2 and 6 $\mu\text{g/g}$ although there was some feeding on diet discs containing lolitrem B at all concentrations. This resulted in significantly delayed growth and fewer larvae pupating at the highest concentration of 6 $\mu\text{g/g}$.

INTRODUCTION

A number of mycotoxins have been isolated from endophyte-infected perennial ryegrass, *Lolium perenne* L. The indole alkaloids, lolitrem A and B, isolated from ryegrass infected with the endophyte, *Acremonium lolii*, are neurotoxic tremorgens responsible for the neuromuscular disorder of grazing stock known as ryegrass staggers (Gallagher *et al* 1982). Lolitrem B is not a feeding deterrent to adults of the graminaceous stem miner, Argentine stem weevil (ASW) *Listronotus bonariensis* (Coleoptera: Curculionidae) at 1 and 5 $\mu\text{g/g}$ but retards larval growth at 5 $\mu\text{g/g}$, (Prestidge and Gallagher 1985). Larvae reared by Prestidge and Gallagher (1988) on 5-20 $\mu\text{g/g}$ lolitrem B developed slowly through all the instars. Another endophyte metabolite, peramine, extracted from *A. lolii* has been found in choice tests to deter feeding by adult ASW at 0.1 $\mu\text{g/g}$ and larvae at 10 $\mu\text{g/g}$ (Dymock *et al* 1988). Unlike larvae reared on diet containing lolitrem B, a significant proportion of larvae reared on 2-25 $\mu\text{g/g}$ peramine in a no-choice situation failed to develop beyond the first instar and eventually died. However, the surviving larvae developed at the same rate as those on control diet.

The aim of this study was to determine whether lolitrem B is a feeding deterrent to ASW larvae and to what extent feeding deterrence could be responsible for the poor growth of larvae on lolitrem B reported by Prestidge and Gallagher (1988). Information on the effect of endophyte-produced mycotoxins on insect feeding and development is necessary for the development of endophyte-infected grasses with insect resistance and low mammalian toxicity.

METHODS

ASW larvae were obtained from eggs laid on endophyte-free perennial ryegrass by captive field-collected weevils. Eggs were surface sterilised in 1% formaldehyde in 70% ethanol with 0.01% Tween 80 as the wetting agent. Single eggs obtained as above were transferred on moist filter paper to 2 ml analyser cups containing two 0.5 ml discs of wheat germ based diet (Malone and Wigley 1989) with and without lolitrem B.

Lolitrem B was extracted and purified from perennial ryegrass seed (Gallagher *et al* 1985). It was dissolved in dichloromethane and adsorbed onto the cellulose powder of the diet to give final concentrations of 0, 0.2, 0.8, 2, 6 $\mu\text{g/g}$. The control and test discs were separated by a horizontal 1.2 cm length nichrome wire embedded in each disc. The position of the test and control discs in the cups relative to the lids was randomised and there were 50 replicates for each lolitrem B concentration. The cups were placed on their sides to remove any gravitational bias and kept at 18 °C with a light regime of 16:8 hours L:D.

After 28 days feeding was scored on a scale of 0-3 (0 = no sign of feeding, 1 = one small area of the disc broken up by feeding, 2 = two-three areas broken up, 3 > = three

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large areas of the disc shredded). The scores were analysed by the Wilcoxon signed rank test (Siegel 1956).

Growth rate was assessed by measuring the head capsule widths of visible larvae at days 18, 28 and 46. When larvae reached the fourth instar they were transferred to analyser cups containing moist vermiculite for pupation. The experiment was continued for 92 days.

RESULTS

Lolitre B deterred ASW larval feeding at 2 and 6 $\mu\text{g/g}$ in the choice test ($P < 0.05$) although there was feeding on both diet discs at all concentrations of lolitre B tests (Table 1).

Larval growth rate in choice tests containing lolitre B at 6 $\mu\text{g/g}$ was significantly lower than that on the control diet at 28 days and thereafter (Table 2). Larval mortality was higher and fewer larvae pupated when reared on a choice of control diet and 6 $\mu\text{g/g}$ lolitre B (Table 2). There did not appear to be any effect of lolitre B on survival of pupae.

TABLE 1: The effect of lolitre B on the feeding of Argentine stem weevil larvae and the total scores on the diet discs after 28 days.

lolitre B ($\mu\text{g/g}$)	Number of larvae			P*
	Prefer lolitre B	Avoid lolitre B	No preference	
6	6	15	7	0.03
2	10	17	7	0.05
0.8	11	15	6	0.47
0.2	10	11	17	0.42
0	12	12	12	0.26

*P = probability that the differences in scores on test and control discs is due to chance (Wilcoxon signed rank test).

TABLE 2: Mean head capsule widths of Argentine stem weevil larvae and larval mortality and number pupating after 92 days when reared on diet discs containing both lolitre B and control diet in choice tests.

lolitre B ($\mu\text{g/g}$)	Mean head capsule width (mm)			% larval mortality	No. pupating	No. emerging
	18 days	28 days	46 days			
6	0.19	0.22	0.34	60.0	8	8
2	0.21	0.34	0.48	32.0	27	21
0.8	0.24	0.41	0.53	32.6	29	25
0.2	0.25	0.42	0.56	10.4	34	27
0	0.20	0.33	0.47	38.3	21	18
LSD*	0.032	0.082	0.070			

*LSD = least significant difference at the 5% level.

DISCUSSION

This study has shown that ASW larvae are deterred from feeding on diets containing lolitre B at 2 and 6 $\mu\text{g/g}$ although there was considerable feeding on both treated and untreated diets. This compared with a strongly deterrent effect ($P = 0.001$) for peramine at 10 $\mu\text{g/g}$ in a similar larval choice test (Dymock *et al* 1988). Levels of lolitre B used in this study are similar to mean levels found in perennial ryegrass (Prestidge and Gallagher 1988). However, lolitre B concentrations in the basal sheath area can exceed 30 $\mu\text{g/g}$ (B. Tapper pers. comm.) and high larval mortality in the basal region of grasses has been observed by Goldson and Vartha (pers. comm.). It is likely that a greater effect on larval behaviour and development would have been recorded if higher rates of lolitre B had been used.

The slowed growth of larvae on diet containing 6 µg/g of lolitrem B is probably due to a combination of both reduced feeding as a result of the deterrent effect observed and possible toxic effects of ingesting the metabolite. In the present choice experiments the relative reduction in larval growth rate was not as marked as that recorded by Prestidge and Gallagher (1988) in a no-choice test mainly because larvae fed on both control and test diet. However, the growth rate of larvae on control diet in this experiment was slower than that recorded on control diet by Prestidge and Gallagher (1988) at similar temperatures. These differences probably reflect differences in sampling methods of larvae for head capsule width measurement.

Adult ASW feeding deterrents such as peramine and the ergopeptine alkaloids (Dymock *et al* 1988) are the most important mechanisms of resistance in ryegrasses infected with *A. lolii*. Their presence ensures that stem weevil larval populations in endophyte-infected plants never become large enough to cause significant plant mortality. However, some oviposition does occur on infected plants. In these cases a combination of the feeding deterrent activity to larvae of both peramine and lolitrem B, accompanied by a reduction in larval growth rate and an increase in mortality, is an important secondary mechanism of resistance.

ACKNOWLEDGEMENTS

We would like to thank Karyn Froud (Entomology Division, DSIR) for skilled technical assistance and Alan Hawkes and Jan Sprosen (MAF Ruakura) for providing lolitrem B samples.

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