LOLITREM B — A STEM WEEVIL TOXIN ISOLATED FROM ACREMONIUM-INFECTED RYEGRASS

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SUMMARY

Lolitre B, a lipophilic complex substituted indole, which is strongly implicated in ryegrass staggers in animals grazing *Acremonium loliae*-infected ryegrass pastures, was tested in bioassays for Argentine stem weevil (*Listronotus bonariensis*) feeding deterrence and toxicity. Adult stem weevil were not deterred from feeding on artificial diet which had lolitre B incorporated at 1 and 5 ppm. When lolitre B was incorporated into the diet at 5 ppm, larval growth rate was significantly reduced and larvae suffered greater mortality compared to feeding on diets without lolitre B. It is possible that intake of lolitre B with food or absorption through integument contact of the tunnelling larvae is responsible for the higher mortality of stem weevil larvae in *A. loliae*-infected ryegrass plants.

INTRODUCTION

The chemical isolation of adult Argentine stem weevil feeding deterrents in *Acremonium loliae*-infected ryegrass (Gaynor *et al* 1983) and in *A. loliae* fungal cultures (Prestidge *et al* 1985a) has highlighted the presence of lipid and water soluble active compounds respectively. The structure of these compounds is at present being determined. From analysis of the properties of these feeding deterrents, it is apparent that they are quite different from the lolitre neurotoxins associated with ryegrass staggers in grazing stock (Gallagher *et al* 1981).

Prestidge *et al* (1985b) have reported that larval survival and feeding activity was not affected by the presence in the diet of a strain of *A. loliae* even when 10% (w:w) of mycelium was included. However when larvae were confined to *A. loliae*-infected ryegrass they died after 2-3 weeks (Barker *et al* 1984) possibly as they tunnelled near the crown of the plant where the mycelium was concentrated; a similar result has been observed by Goldson (pers comm). This suggested the presence of toxic compounds in the plant, resulting in some way from interactions between the plant and fungus.

Although alkaloids such as perloline and halostachine have long been known to be present in ryegrass plants (eg. Aasen *et al* 1969), we were interested in the possible involvement of the potent lolitre neurotoxins. The structure of the major neurotoxin in *A. loliae*-infected ryegrass, lolitre B, has now been completely elucidated (Gallagher *et al* 1984). It is a highly lipophilic complex substituted indole compound with a molecular weight of 685 and chemical formula $C_{42}H_{57}NO_7$. In terms of possible toxicity to insects, the lipid soluble property would enhance the likelihood of neurotoxicity (eg. Beeman 1982) especially when insects are feeding near the base of the plant where the neurotoxin levels are highest (Gallagher *et al* 1984). Gallagher *et al* (1985) have now developed a sensitive, quantitative method of extraction and analysis of the lolitre B neurotoxin.

The aim of the work reported in this paper was to test lolitre B, extracted from *A. loliae*-infected ryegrass, for Argentine stem weevil feeding deterrence and toxicity.

METHODS

Lolitre B was extracted and purified from Ellett ryegrass by the methods of Gallagher *et al* (1985).

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Adult bioassay

Pure lolitrem B was dissolved in 2 ml of chloroform and incorporated into an agar based artificial diet. Discs of diet with lolitrem B or without lolitrem B were offered in choice tests to reproductive adult stem weevil which had been field collected and starved for 24 h. For comparison, a 1% supernatant of cultured *A. loliae* isolated from 'Grasslands Ariki' ryegrass was also included as a treatment. Forty weevils/treatment were used, confined individually to petri dishes with moist filter paper at 20°C and 16:8 h (L:D) photoperiod. Adults were left to feed for 48 h after which time feeding activity on the discs was scored (Prestidge *et al* 1985a).

Larval bioassay

First instar stem weevil larvae, hatched from field collected eggs, were transferred onto discs of an artificial diet in sealed microtitre vials in paired experiments using treated (lolitrem B added) and untreated (no lolitrem B) discs. Thirty larvae were used per treatment and each experiment was repeated once. The experiment was subsampled at regular intervals and larval survival, head width and body length were measured.

Lolitrem B was tested at 1 and 5 ppm. Data from adult and larval bioassays were tested for statistical significance by the Wilcoxon signed-rank test (Siegel 1956) and students "t" test respectively.

RESULTS AND DISCUSSION

Adult stem weevil were not outwardly affected by the presence of lolitrem B in the diet and showed no signs of being deterred from feeding on the treated disc (Table 1). In comparison the 1% supernatant of cultured *A. loliae* was highly active and deterred adults from feeding which showed that the stem weevil population was capable of detecting feeding deterrents (Prestidge *et al* 1985a). Although the experiments were completed after 48 h, there were no dead adults suggesting that the material was not acutely toxic at the levels tested. Small quantities of chloroform do not affect the feeding behaviour of adult stem weevil (Prestidge *et al* 1985a).

TABLE 1: The percentage of weevils feeding on discs of artificial diet with (treated) and without (untreated) lolitrem B or culture supernatant added.

Lolitrem B	% weevils showing feeding				
	Treated disc	Untreated disc	Sign.	No pref.	No feeding
1 ppm	28	35	ns	25	12
5 ppm	40	33	ns	15	12
1% supernatant	7	69	***	11	13

ns Not significant

*** $p < 0.001$

At 1 ppm, lolitrem B in the diet had no significant effect on larval growth rate or survival (Table 2). However at 5 ppm, larvae took longer to reach prepupal size and suffered greater mortality. Neither of the lolitrem B concentrations appeared to deter larval feeding. The addition of 2 ml of chloroform to the diet had no effect on larval growth and survival.

Levels of lolitrem B commonly found in *A. loliae*-infected ryegrass vary according to the plant and the season. During summer and autumn, levels often vary from 1-5 ppm, with highest levels being found near the base of the plant in the basal leaf sheath zone. Levels of lolitrem B in some lines of infected ryegrass have been found to exceed 8 ppm (Gallagher unpubl. data). It is therefore possible that intake of lolitrem B, either by ingestion with food or through integument contact of the tunnelling larvae, is responsible for the higher mortality of stem weevil larvae in *A. loliae*-infected ryegrass plants. In an experiment where the toxin has been incorporated into an artificial diet, the latter route of toxin absorption could be especially important. Lolitrem B may be a major cause of both ryegrass staggers and the resistance to Argentine stem weevil associated with *A. loliae* infection, but not of adult feeding deterrence (Gaynor *et al*

TABLE 2: Growth and mortality of stem weevil larvae feeding on artificial diets with and without incorporation of lolitrem B at 1 and 5 ppm.

No. days feeding	Head width (mm)		Body length (mm)		Cumulative mortality(%)	
	Untreated	1 ppm	Untreated	1ppm	Untreated	1 ppm
13	0.43	0.43	3.66	3.64	3	7
21	0.53	0.53	4.92	4.54	7	7
27	0.55	0.51	4.76	5.76	7	7
	Untreated	5 ppm	Untreated	5 ppm	Untreated	5 ppm
16	0.51	0.33***	4.17	2.21***	10	20
24	0.56	0.37***	5.00	2.30***	13	40*
30	0.53	0.49	4.50	4.07	13	43*

* $p < 0.05$ *** $p < 0.001$

1983, Prestidge *et al* 1985a), which is the more important mechanism of resistance to stem weevil as it ensures eggs are not oviposited on *A. loliae*-infected ryegrass.

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