EFFECT OF SELECTED ISOLATES OF *ACREMONIUM* ENDOPHYTES ON ADULT BLACK BEETLE (*HETERONYCHUS ARATOR*) FEEDING

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SUMMARY

The role of lolitrem B, peramine, the ergopeptines (including ergovaline) and the lolines, in the feeding behaviour of adult black beetle (Coleoptera: Scarabaeidae) was investigated using five novel perennial ryegrass/*Acremonium* associations. Feeding preference studies were conducted by adding single adult black beetles to pots containing tillers of both endophyte-infected and endophyte-free ryegrass plants. All five ryegrass/*Acremonium* associations were significantly less damaged than the endophyte-free controls, regardless of their known alkaloid spectra. These studies indicated that in some of the associations, lolitrem B, peramine and ergovaline, as well as the loline alkaloids, were not essential to deter the feeding of adult black beetle.
Keywords: black beetle, endophyte, perennial ryegrass, alkaloids.

INTRODUCTION

The presence of the endophytic fungus *Acremonium lolii* Latch, Christensen and Samuels has been shown to deter adult black beetle (*Heteronychus arator* F.), a pest of northern North Island pastures, from feeding on perennial ryegrass (*Lolium perenne* L.) host plants (Ball and Prestidge 1992). Studies in which purified *Acremonium* endophyte-associated alkaloids were incorporated into artificial diets indicated that ergotamine (at 5.0 µg/g) was an adult black beetle feeding deterrent (Ball and Prestidge 1993). Peramine, the principle Argentine stem weevil (*Listronotus bonariensis* Kuschel) feeding deterrent (Gaynor and Rowan 1985), lolitrem B, the alkaloid largely responsible for causing ryegrass staggers in grazing animals (Gallagher *et al.* 1981), and paxilline did not deter adult black beetle from feeding. This result suggested that ergopeptine alkaloids in general (including ergovaline which is the most prevalent ergopeptine alkaloid in *A. lolii*-infected perennial ryegrass) may be important in maintaining the resistance against black beetle.

With the availability of *Acremonium* isolates producing different alkaloid spectra in host grasses (Christensen *et al.* 1991, 1993), experiments were performed using plants artificially infected with five different *Acremonium* strains to determine whether peramine, lolitrem B and ergovaline were required to maintain black beetle resistance.

METHODS

Feeding bioassays testing the effects of five different *Acremonium* endophyte strains (Lp5, Lp14, Lp17, Tf13 and Tf16) in 'Grasslands Nui' perennial ryegrass on adult black beetle feeding were performed. Analysis of lolitrem B, peramine, ergovaline, total ergot alkaloid and loline alkaloid content has shown that the chemical profile of Nui plants infected with these endophytes is strain dependent (Christensen *et al.* 1993, Christensen unpubl. data, Garthwaite unpubl. data) (Table 1). Isolates Tf13 and Tf16 were derived from tall fescue (*Festuca arundinacea* Schreb.), the endophyte of which is usually referred to as *Acremonium coenophialum* Morgan-Jones and Gams. However, Tf13 and Tf16 are not classified as *A. coenophialum*, but are recognised as belonging to the *F. arundinacea* taxonomic groupings (FaTG) two and three respectively (Christensen *et al.* 1993). Endophyte strains Lp5, Lp14 and Lp17 were derived from perennial ryegrass and are all classified as *A. lolii.*

TABLE 1: *Acremonium* endophyte strains used in the black beetle feeding study and their alkaloid profiles when in associations with "Nui" perennial ryegrass plants.

Strain	Taxon[1]	lolitrem B	peramine	ergopeptines	ergovaline	lolines
Lp5	*A. lolii*	+	+	+	+	-
Lp14	*A. lolii*	-	-	-(?)	-	-
Lp17	*A. lolii*	-	-	-(?)	-	-
Tf13	FaTG-2[2]	-	-	+	+	-
Tf16	FaTG-3[3]	-	+	+(?)	-	-(?)

[1] Christensen *et al.* 1993.
[2] *Acremonium* taxon, "*Festuca arundinacea* taxonomic grouping 2"
[3] *Acremonium* taxon, "*Festuca arundinacea* taxonomic grouping 3"
(?) Not consistently detected or present in trace amounts.

Mature ryegrass plants, the endophyte status of which were verified by microscopic examination of stained leaf sheaths prior to each trial, were used in all feeding tests. Feeding tests were of the choice type and were performed in the laboratory. On day zero of each test, four tillers from each of the two test plants to be compared were planted into a pot (90 x 80 mm diameter) which was filled to within 2 cm of the top with sieved Horotiu sandy loam soil. This was replicated 7 to 10 times.

Six choice feeding tests comparing different plant combinations were performed. Tests 1, 2, 3 and 5 were performed in the spring of 1993, while tests 4 and 6 were performed in the autumn of 1994.

Test 1: Ten Nui perennial ryegrass plants (five genotypes) infected with strain Lp5 were compared with ten endophyte-free Nui plants (ten genotypes).

Test 2: Seven Nui plants (seven genotypes) infected with strain Tf13 were compared with seven endophyte-free Nui plants (seven genotypes).

Test 3: Ten Nui plants (five genotypes) infected with strain Lp5 were compared with ten Nui plants (five genotypes) infected with strain Tf13.

Test 4: Nine Nui plants (three genotypes) infected with strain Tf16 were compared with nine endophyte-free Nui plants (six genotypes).

Test 5: Eight Nui plants (four genotypes) infected with strain Lp14 were compared with eight endophyte-free Nui plants (eight genotypes).

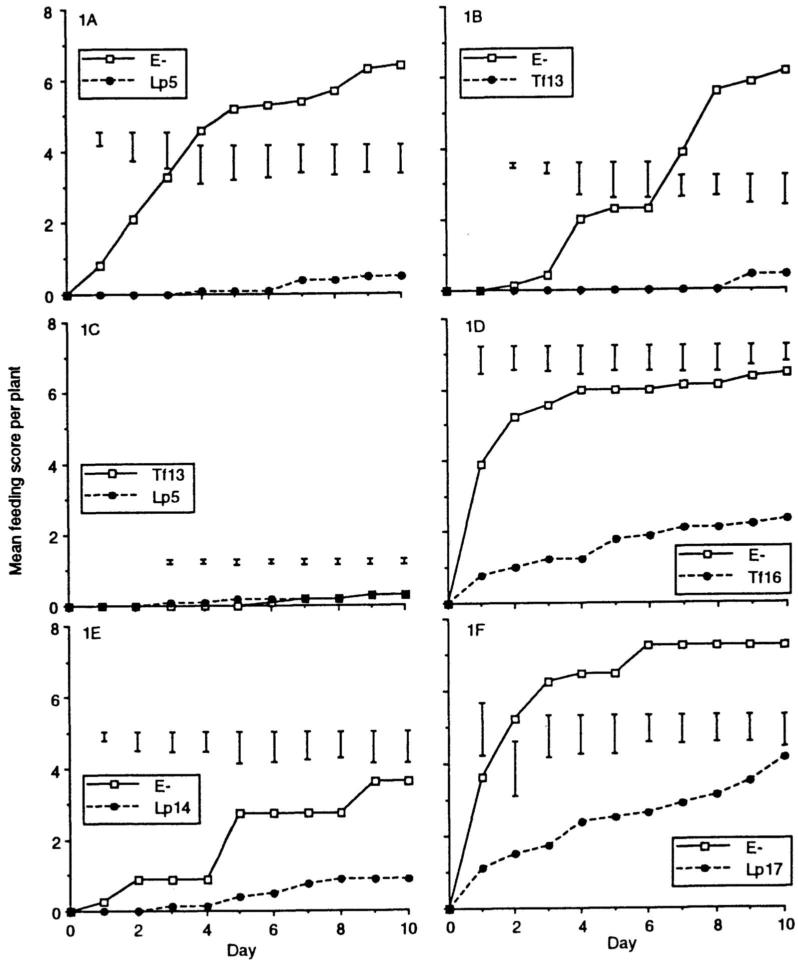
Test 6: Eight Nui plants (three genotypes) infected with strain Lp17 were compared with eight endophyte-free Nui plants (six genotypes).

After planting, each pot was seeded with one adult black beetle. Where possible, equal numbers of males and females were used for each test. Each pot was covered with an inverted clear plastic container with ventilation holes to prevent the black beetle from escaping. The number of healthy, black beetle damaged and black beetle killed tillers on each plant was assessed every day for 10 days. Tillers were scored according to the damage sustained. Tillers which were killed were given a score of 2. Tillers which were damaged were given a score of 1. Healthy tillers were given a score of zero. Daily feeding scores for each plant were calculated by adding together the feeding scores for the four tillers of each plant. Thus the highest feeding score possible for each plant was 8. Daily mean feeding scores per plant for all replicate plants in each test were calculated. Mean feeding scores in each experiment were analyzed using paired difference *t* tests.

RESULTS

Test 1: Two days after black beetle were introduced, mean feeding scores were significantly ($P<0.05$) higher in the endophyte-free plants than the Lp5-infected plants and remained so for the rest of the trial (Fig. 1A). From day 5 onwards the difference in the mean feeding scores was significant at $P<0.01$. Mean feeding scores in the Lp5-infected plants remained low for the duration of the trial. On day ten, the mean feeding score in Lp5-infected plants was 0.5, while the mean feeding score in endophyte-free plants was 6.4.

Test 2: From day 7 onwards, tiller damage and tiller mortality due to adult black beetle feeding was significantly ($P<0.001$) higher in endophyte-free Nui than in Tf13-infected Nui (Fig. 1B). After 10 days, the mean feeding score in endophyte-free plants was 6.1 compared to 0.4 in Tf13-infected plants.



Figures 1: A-F. Mean feeding scores from choice tests investigating black beetle resistance in Nui plants infected with five different *Acremonium* strains (E- = endophyte-free plants). (Bars represent S.E.D).

Test 3: Mean feeding scores in Lp5-infected Nui and Tf13-infected Nui were low throughout the 10 days of the trial and at no point were they significantly different (Fig. 1C). On day 10, the mean feeding score in both Lp5-infected and Tf13-infected Nui was 0.3.

Test 4: On days 1 and 2 of the trial, mean feeding scores in endophyte-free Nui plants increased sharply to a high level, while only a minor increase was recorded in Tf16-infected plants (Fig. 1D). Throughout the entire trial, feeding scores were significantly ($P<0.01$ on day 1, $P<0.001$ from day 2 onwards) higher in endophyte-free Nui plants compared to plants infected with isolate Tf16. On the final day of the trial, feeding scores for the endophyte-free and Tf16-infected plants were 6.4 and 2.3 respectively.

Test 5: Mean feeding scores in endophyte-free plants increased gradually and were consistently higher than mean feeding scores in Lp14-infected plants (Fig. 1E). From day 5 onwards, mean feeding scores were significantly ($P<0.05$) greater in endophyte-free plants compared to Lp14-infected plants. On day 10, the mean feeding score in endophyte-free Nui was 3.6 compared to 0.9 in Lp14-infected Nui.

Test 6: From day 1 to day 3, mean feeding scores increased steadily in endophyte-free plants, and reached their highest level on day 6 (Fig. 1F). Mean feeding scores in Lp17-infected plants increased steadily throughout the trial, although they were consistently lower (significantly ($P<0.05$ - $P<0.001$) so from day 2 onwards) than feeding scores from the endophyte-free plants. On day 10 of the trial, feeding scores for the endophyte-free and Lp17-infected plants were 7.3 and 4.1 respectively.

DISCUSSION

All five strains of *Acremonium* endophyte conferred resistance to perennial ryegrass against adult black beetle (Figs. 1A-F). Thus, *Acremonium* endophytes derived from tall fescue conferred resistance to perennial ryegrass host plants against adult black beetle attack (Figs. 1B-D) as was previously found with *A. lolii*-infected perennial ryegrass (Ball and Prestidge 1992).

The black beetle resistance observed in Nui plants infected with *Acremonium* isolates Tf13, Tf16, Lp14 and Lp17 (Figs. 1B, 1D, 1E and 1F respectively) occurred in the absence of detectable levels of peramine and lolitrem B. Also, a direct comparison indicated that Nui plants infected with *Acremonium* strain Tf13 (which contained ergovaline but no detectable peramine or lolitrem B) were no less resistant to adult black beetle than Nui plants infected with strain Lp5 (which contained peramine, lolitrem B and ergovaline) (Fig. 1C). These results strongly suggest that peramine and lolitrem B are not required for endophyte-mediated adult black beetle resistance in perennial ryegrass. This contention is supported by the results of Ball and Prestidge (1993) who found that peramine and lolitrem B incorporated into artificial diets had no effect on adult black beetle feeding.

Ergotamine incorporated into artificial diets has been shown to strongly deter adult black beetle from feeding (Ball and Prestidge 1993) indicating that ergovaline or other ergopeptine alkaloids may be important in maintaining resistance to black beetle in plants infected with isolates Lp5 and Tf13. However, plants infected with *Acremonium* isolates Tf16, Lp14 and Lp17 which strongly deterred adult black beetle from feeding (Figs. 1D-F), do not produce detectable amounts of ergovaline. Furthermore, ergot alkaloids are present only in trace amounts in these associations, if at all (Table 1). Trace quantities of ergot alkaloids may be sufficient to deter the black beetle from feeding. However, compounds from a completely different chemical group (or groups) may instead be involved in the resistance conferred by these isolates. Loline alkaloids are unlikely to be a candidate as they were not produced in ryegrass infected with *Acremonium* isolates LP14 and LP17 and are only rarely present in detectable quantities in Tf16-infected Nui plants (Christensen unpubl. data).

The results of this study indicate that it may be possible to incorporate strains of *Acremonium* endophytes into cultivars of perennial ryegrass which do not produce the known mammalian toxins lolitrem B and ergovaline, but which provide protection from important pasture pests such as Argentine stem weevil and black beetle.

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REFERENCES

Ball, O.J-P. and Prestidge, R.A., 1992. The effect of the endophytic fungus *Acremonium lolii* on adult black beetle (*Heteronychus arator*) feeding. *Proc. 45th N.Z. Plant Protection Conf.*: 201-204.

Ball, O.J-P. and Prestidge, R.A., 1993. The use of the endophytic fungus *Acremonium lolii* as a biological control agent of black beetle, *Heteronychus arator* (Coleoptera: Scarabaeidae). *Proc. 6th Australasian Conf. Grassl. Invert. Ecol.*: 283-289.

Christensen, M.J., Latch, G.C.M. and Tapper, B.A., 1991. Variation within isolates of *Acremonium* endophytes from perennial rye-grasses. *Mycol. Res. 95*: 918-923.

Christensen, M.J., Leuchtmann, A., Rowan, D.D. and Tapper, B.A., 1993. Taxonomy of *Acremonium* endophytes of tall fescue (*Festuca arundinacea*), meadow fescue (*F. pratensis*), and perennial rye-grass (*Lolium perenne*). *Mycol. Res. 97*: 1083-1092.

Gallagher, R.T., White, E.P. and Mortimer, P.H., 1981. Ryegrass staggers: isolation of potent neurotoxins lolitrem A and lolitrem B from staggers producing pastures. *N.Z. Vet. J. 29*: 189-190.

Gaynor, D.L. and Rowan, D.D., 1985. Peramine - An Argentine stem weevil feeding deterrent from endophytic-infected ryegrass. *Proc. 4th Australasian Conf. Grassl. Invert. Ecol.*: 338-343.