SEASONAL LEVELS OF PERAMINE AND LOLITREM B IN *ACREMONIUM LOLII*-INFECTED PERENNIAL RYEGRASS

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SUMMARY

Concentrations of lolitrem B and peramine in monthly samples of *Acremonium lolii*-infected perennial ryegrass were determined by HPLC techniques. Mean lolitrem B levels increased from an August low to peak at 5 ug/g in February. Mean peramine levels peaked at 24 ug/g in January. Variation in monthly peramine concentration between plants was greater than in lolitrem B. Relative concentrations of peramine and lolitrem B in ryegrass plants were maintained throughout the year. The possible causes of the changes in lolitrem B and peramine concentrations and their implications for insect resistance are discussed.

Keywords: Argentine stem weevil, alkaloids, endophyte

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is the most widely used pasture grass in New Zealand. The presence of a Clavicipitaceous fungal endophyte (*Acremonium lolii* Latch, Christensen and Samuels) confers resistance to perennial ryegrass (PRG) against Argentine stem weevil (ASW) (*Listronotus bonariensis* (Kuschel)) attack (Prestidge *et al* 1982). Peramine, an adult ASW feeding deterrent produced by the fungus was later identified (Rowan and Gaynor 1986). Fletcher and Harvey (1981) reported that the occurrence of ryegrass staggers was associated with the presence of *A. lolii*-infected PRG in the pasture. Gallagher *et al* (1981) identified lolitrems, a group of tremor-inducing alkaloids, from ryegrass staggers prone pastures. Lolitrem B, also produced by the fungus (M.E. di Menna and N.R. Towers, pers. comm.) is thought to account for most of the ryegrass staggers symptoms.

The purpose of this study was to monitor seasonal concentrations of lolitrem B and peramine in *A. lolii*-infected PRG as part of an investigation into the environmental factors which may be responsible for alkaloid production and retention in the plant.

METHODS

Twenty mature PRG plants obtained from a variety of North Island habitats, were grown outdoors at Ruakura, Hamilton. The plants were taken from pastures which were at least 30 years old and were selected for high or low summer levels of lolitrem B (Wedderburn *et al* 1989). Plants were grown approximately 50 cm apart in Horotiu sandy loam soil which was kept weed free by hand weeding. Plants were trimmed and watered as necessary, but were allowed to set seed.

During 1990, samples of approximately 20 to 30 tillers were taken from each plant at monthly intervals, by harvesting at ground level with a scalpel. Regrowth from the previous months sampling was not sampled and dead tillers were discarded. Grass material was washed in tap water to remove soil debris, dried at 60 °C for 40 hours and finely ground. Samples were stored at -20 °C until chemical analysis.

Lolitrem B and peramine were analysed by high performance liquid chromatography (HPLC), lolitrem B by the method of Gallagher *et al* (1985), and peramine by a modification of a method supplied by B.A. Tapper (D.S.I.R., Palmerston North) using extraction by aqueous isopropanol, ion exchange clean-up, and analysis on a silica column.

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Climate data were obtained from a weather station situated approximately 200 m from the site.

RESULTS AND DISCUSSION

Lolitre B concentrations (Fig. 1) were highest during summer and lowest during winter. Winter levels were approximately one tenth of those in the summer. Mean lolitre B levels were significantly correlated to mean monthly grass minimum temperatures ($r^2=0.81$) (Figs. 1 and 2). These results are in general agreement with those of Prestidge and Gallagher (1988), although in their study, lolitre B levels peaked approximately 2 months later. This may be due to differences in environmental conditions during the two trials. Temperature profiles during the respective trials, however, were very similar. Prestidge and Gallagher (1988) found that lolitre B levels were highest during months with low rainfall. Similarly, Kennedy and Bush (1983) found that loline alkaloid concentrations in *Acremonium coenophialum*-infected tall fescue (*Festuca arundinacea*) increased significantly during periods of water stress.

Fig. 1: Mean monthly lolitre B and peramine concentrations in *A. lolii*-infected PRG during 1990 (vertical bars represent SEM).

Fig. 2: Total monthly rainfall (■) and mean monthly grass minimum temperature (□—□) for 1990.

Lolitre B concentrations in the present study also peaked during periods of low rainfall (Figs. 1 and 2). Whether this was a direct effect of moisture stress is uncertain, however, as the plants were irrigated over summer and did not appear to be stressed.

di Menna and Waller (1986) showed that counts of *A. lolii* hyphae in the leaf sheath were highest in summer and lowest during the winter which correlates with the observed seasonality in production of lolitre B in the present study. They also suggested, however, that moisture stress may lead to a reduction in the number of *A. lolii* hyphae, whereas lolitre B levels appeared to increase during such periods.

During the summer and winter, lolitre B levels in individual plants ranged from 0.91 ug/g to 9.75 ug/g and 0.09 ug/g to 3.04 ug/g respectively. Plants could be characterised by their lolitre B concentration as they generally maintained relative levels throughout the year (Fig. 3). The genetic characteristics of both the fungus and the PRG, and the concentration of *A. lolii* mycelium in the plant, may be important factors in determining the relative level of lolitre B in a plant.

Fig. 3: Monthly lolitre B and peramine concentrations from a representative high alkaloid plant (■—■ = lolitre B, *--* = peramine) and low alkaloid plant (+--+ = lolitre B, □—□ = peramine).

Prestidge and Gallagher (1985) reported that adult ASW were not deterred from feeding on artificial diets with lolitre B concentrations of 5 ug/g (wet weight) (this equates to approximately 40-50 ug/g dry weight (D.D. Rowan, pers. comm.)). In the current study, a lolitre B concentration of 5 ug/g (dry weight) for individual plants was considered high during the summer. It is unlikely therefore that lolitre B has a role in deterring natural infestations of adult ASW. However, ASW larval growth rates and survival are significantly reduced at 5 ug/g (wet weight) (Prestidge and Gallagher 1985), and Dymock *et al* (1989) found that 2 ug/g (wet weight) of lolitre B incorporated into artificial diets acted as a feeding deterrent to ASW larvae. Lolitre B concentrations in the whole plant preparations recorded in this study were therefore probably too low to adversely affect ASW larvae. However, lolitre B may still provide resistance against larval ASW attack as concentrations in the basal leaf sheath area are higher than those in the leaf blade (Gallagher *et al* 1987).

Inter-plant variation in seasonal peramine levels was considerable, resulting in a less discrete seasonal pattern. Summer and winter levels of peramine in individual PRG plants ranged between 2.5 ug/g — 41.6 ug/g and 2.9 ug/g — 36.1 ug/g respectively. Large monthly fluctuations in peramine concentration within individual plants were also recorded. It is possible that the variation in the data was due to the unequal distribution of peramine within each plant. However, treatments for all plants were identical and the large sample sizes used should have assisted in reducing this problem.

The trimming regime may also account for some of the variation. Fannin *et al* (1990) found that the highest concentrations of peramine occur in the leaf blade. The concentration of peramine in the different tissues of the grass was not monitored in this study.

Compared to lolitrem B, mean peramine levels were relatively stable throughout the year although concentrations in winter were significantly ($P < 0.01$) lower than summer and autumn levels (Fig. 1). Unlike lolitrem B, mean peramine levels decreased significantly ($P < 0.01$) between January and March, and rose in April ($P < 0.05$). Spring peramine levels were only slightly higher than those in winter ($0.1 > P > 0.05$). Concentrations of peramine in December were significantly ($P < 0.05$) higher than in November and lower than in the previous January ($P < 0.01$).

Mean peramine levels were significantly correlated with changes in mean monthly grass minimum temperature ($r^2 = 0.34$) (Figs. 1 and 2). Compared to lolitrem B, however, the correlation was not strong. It is therefore uncertain whether peramine levels correlate with the seasonality of *A. lolii* observed by di Menna and Waller (1986). The variable response of individual plants suggests that peramine synthesis may be influenced by many physical, biotic, and genetic factors acting together, rather than by one or two major climatic factors.

As with lolitrem B, relative concentrations of peramine in individual ryegrasses were also maintained throughout the year.

Dymock *et al* (1988) showed that ASW larval mortality was increased when feeding on diets containing 2 $\mu\text{g/g}$ (wet weight) of peramine. Adult ASW are deterred from feeding by peramine levels as low as 0.1 $\mu\text{g/g}$ (wet weight) (Dymock *et al* 1988) although Popay (1990) found that 10 $\mu\text{g/g}$ (wet weight) was required to reduce feeding in a no choice situation. In these experiments, the concentration of peramine in the diet was calculated on a wet weight basis. Analysis of peramine concentrations in PRG in this study was performed on dry matter. It is therefore difficult to compare the effects on ASW of naturally occurring peramine in *A. lolii*-infected PRG with peramine which has been artificially added to diets. However, peramine concentrations of 10 $\mu\text{g/g}$ (dry weight) in winter were common, indicating that a degree of weevil protection may be maintained throughout the year in *A. lolii*-infected PRG.

Mean lolitrem B and peramine levels were positively correlated ($r^2 = 0.74$) (Fig. 4). This suggests that the synthesis of the two alkaloids could be linked by one or more common factors, the concentration of *A. lolii* mycelium in the plant being an obvious possibility. It also suggests, however, that an *A. lolii*-infected PRG plant which is high in peramine but low in lolitrem B may occur infrequently in natural populations.

Fig. 4: Relationship between the mean yearly concentrations of lolitrem B and peramine in endophyte-infected PRG.

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