ACREMONIUM LOLII, ERGOVALINE AND PERAMINE PRODUCTION IN ENDOPHYTE-INFECTED PERENNIAL RYEGRASS

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ABSTRACT

The concentrations of the endophytic fungus *Acremonium lolii* and two associated alkaloids, ergovaline and peramine, were monitored in eight perennial ryegrass/endophyte associations in the spring, summer, autumn and winter of 1992. Mean *A. lolii* and peramine concentrations varied according to the season but ergovaline concentrations were constant. *A. lolii*-infected plants could be characterised as high, medium or low ergovaline-producing plants, as the plants maintained similar relative levels of ergovaline throughout the sampling period. Yearly mean ergovaline and peramine concentrations, and peramine and *A. lolii* concentrations were correlated, but *A. lolii* and ergovaline concentrations were correlated only in spring. These results are discussed in relation to the use of *A. lolii* in pastures.

Keywords: endophyte, alkaloids, animal health, insect resistance, *Lolium perenne*

INTRODUCTION

The endophyte of perennial ryegrass (*Lolium perenne* L.) *Acremonium lolii* Latch, Christensen and Samuels, is of great significance to New Zealand agriculture. In addition to causing animal health problems (eg. ryegrass staggers, heat stress, hormone imbalance (Fletcher 1993)) in livestock, the presence of *A. lolii* confers resistance to the infected host grass against several important insect pasture pests including Argentine stem weevil (*Listronotus bonariensis* (Kuschel)) and black beetle (*Heteronychus arator* F.) (Prestidge and Ball 1993). These effects are due to the production of a number of different alkaloids by the endophyte.

Research to date has concentrated (by selection of naturally occurring strains or by genetic engineering) on developing endophyte/perennial ryegrass associations which produce the "beneficial" alkaloids associated with insect deterrence (eg. peramine), but which do not produce the "detrimental" alkaloids associated with livestock problems (eg. lolitrem B and ergovaline). Using this approach, care must be taken to ensure that the alkaloids which are known primarily as the cause of the livestock disorders, are not also important in conferring resistance to the host plant against present and unknown insect pasture pests. There is evidence indicating that both lolitrem B and ergovaline are active against two insect pests (Popay and Rowan 1993).

Previous studies (Ball *et al.* 1995) have shown that interrelationships exist between *A. lolii* concentration and the concentration of peramine and lolitrem B in perennial ryegrass. This paper reports on an experiment using natural plant/endophyte associations where the levels of *A. lolii* mycelium, peramine and ergovaline were monitored.

METHODS

Eight genetically distinct *A. lolii*-infected, and one endophyte-free, perennial ryegrass plants were used in the study. The *A. lolii*-infected plants were progeny of paired crosses performed between plants, collected from a variety of North Island habitats, which had been characterised according to their lolitrem B content. The

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progeny plants used encompassed a wide range of lolitrem B and *A. lolii* concentrations. The endophyte-free plant (cv. Yatsyn 1) was included as a control plant for the *A. lolii* ELISA. Each of the nine plants was split into four clones which were planted approximately 50 cm apart into Horotiu sandy loam soil on 9/7/91 and maintained outdoors at the Ruakura Agricultural Research Centre, Hamilton, New Zealand. All plants were microscopically examined seasonally for infection with *A. lolii* using the techniques described in Ball *et al.* (1991). Plants were exposed to rainfall and received no artificial irrigation. All plants were trimmed to 8 cm at approximately monthly intervals.

On 31/1/92 (summer), 2/5/92 (autumn), 2/8/92 (winter) and 2/11/92 (spring), shoot material from one clone of each plant genotype was harvested by cutting all the vegetative tillers immediately above the crown with a scalpel. Harvested shoot material was immediately frozen at -20°C, and then freeze-dried, and ground to a fine

FIGURE 1: Mean *A. lolii* concentrations in eight wildtype perennial ryegrass plants sampled in the summer (January), autumn (May), winter (August) and spring (November) of 1992. (Bars represent SEM).

FIGURE 2: Mean peramine and ergovaline concentrations in eight wildtype *A. lolii*-infected perennial ryegrass plants sampled in the summer (January), autumn (May), winter (August) and spring (November) of 1992. (Bars represent SEM).

powder. The samples were analysed for peramine content using HPLC and *A. lolii* content using ELISA (enzyme-linked immunosorbent assay) as described by Ball *et al.* (1995), and for ergovaline content by reverse phase HPLC (Barker *et al.* 1993). The reported ergovaline levels in the plant are the sum of ergovaline and its epimer ergovalinine.

RESULTS

Mean *A. lolii* concentrations were highest in the summer and lowest in the winter harvests (Fig. 1). Mean peramine concentrations peaked in the autumn harvest and were lowest in the spring harvest (Fig. 2). No variation in mean ergovaline concentrations over the four sampling dates was observed (Fig. 2).

Although inter-plant variation was present, plants could generally be characterised as low, medium or high ergovaline-producing plants according to their ergovaline

FIGURE 3: Ergovaline concentrations in representative high, medium and low ergovaline-containing *A. lolii*-infected perennial ryegrass plants sampled in the summer (January), autumn (May), winter (August) and spring (November) of 1992.

FIGURE 4: Relationship between yearly mean ergovaline and peramine concentrations in eight wildtype *A. lolii*-infected perennial ryegrass plants.

content, as they maintained similar relative concentrations throughout the four sampling times. Representative examples of high, medium and low ergovaline-producing plants are given in Fig. 3.

Ergovaline concentrations for the four sample dates for each plant genotype were significantly correlated ($P < 0.05$) with peramine concentrations (Fig. 4). Also, for each plant yearly mean peramine concentrations, were significantly correlated ($P < 0.001$) with yearly mean *A. lolii* concentrations (Fig. 5). Although yearly mean ergovaline

FIGURE 5: Relationships between yearly mean peramine and yearly mean *A. lolii* concentrations; and ergovaline and *A. lolii* concentrations in the spring harvest (November) in eight wildtype perennial ryegrass plants.

concentrations, were not significantly correlated with yearly mean *A. lolii* concentrations, they were significantly correlated ($P < 0.05$) in the spring harvest (Fig. 5).

DISCUSSION

With respect to seasonal changes in peramine and *A. lolii* levels, the results of the present study agree with those of a previous and more detailed study with different, but related (parent) plants (Ball *et al.* 1995). Although there were only a limited number of sampling dates in the present experiment making it difficult to detect seasonal effects, in both studies the *A. lolii* concentrations peaked in summer, while peramine concentrations were highest during the summer and autumn periods. It is therefore interesting to note that ergovaline concentrations did not fluctuate over the four harvest dates. However Woodburn *et al.* (1993) reported that ergovaline levels in Australian pasture samples were lower in winter, and a similar winter decline has been observed for pasture in New Zealand (L. Fletcher, G. Lane, pers. comm.).

The consistency of relative concentrations of ergovaline in the different plants over time indicates that perennial ryegrass plants with a natural (wildtype) endophyte infection can be characterised as high, intermediate or low ergovaline-producing plants. This has also previously been shown for peramine and lolitrem B (Ball *et al.* 1995). With the correlation found here between ergovaline levels and peramine levels, and the correlation already established (Ball *et al.* 1991) between peramine and lolitrem B levels, high, intermediate or low alkaloid plant-endophyte associations can be identified. A correlation between levels of peramine and lolitrem B in endophyte-infected perennial ryegrass plants, and the concentration of *A. lolii* mycelium in plants has been reported (Ball *et al.* 1995) suggesting the level of infection within a plant may be the primary determinant of alkaloid levels. A highly significant correlation is again evident between yearly mean peramine and *A. lolii* concentrations in plants in the current study. While the correlation between ergovaline and *A. lolii* concentrations in

this experiment is significant only for the spring harvest, the correlation of yearly mean ergovaline and peramine levels suggests there is a common factor underlying differences between alkaloid concentrations in the plants, which is likely to be differences in the level of *A. lolii* mycelium. These results are interesting in the light of recent work indicating that no correlation existed between peramine and ergovaline concentrations in endophyte-infected tall fescue (Roylance *et al.* 1994).

This study has presented evidence indicating that ergovaline, peramine and *A. lolii* levels in perennial ryegrass plants with a natural wildtype endophyte infection are proportionally related, extending the finding of Ball *et al.* (1995) that levels of peramine, lolitrem B and *A. lolii* are correlated. While this correlation has been found for natural ryegrass-endophyte associations from a diverse range of North Island habitats in New Zealand, it may not be assumed to apply generally, particularly for novel associations. The findings indicate that wildtype associations containing low levels of *A. lolii* mycelium are likely to contain only low levels of alkaloids. While the comparative sensitivity of insect pests and livestock to endophyte-produced alkaloids is not yet clearly defined, it may be possible to select low-alkaloid ryegrass-endophyte associations for which animal health problems are eliminated or significantly reduced while insect resistance is maintained. The natural occurrence of low alkaloid ryegrass-endophyte associations encourages the view that insect resistance based on the presence of a cocktail of alkaloids, albeit at a reduced level, may be robust.

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REFERENCES

- Ball, O.J-P., Prestidge, R.A. and Sprosen, J.M., 1995. Interrelationships between *Acremonium lolii*, peramine and lolitrem B in perennial ryegrass. *Appl. Env. Micr.* 61: 1527-1533
- Ball, O.J-P., Prestidge, R.A., Sprosen, J.M. and Lauren, D.R., 1991. Seasonal levels of peramine and lolitrem B in *Acremonium lolii*-infected perennial ryegrass. *Proc. N.Z. Weed and Pest Control Conf.* 44: 176-180
- Barker, D.J., Davies, E., Lane, G.A., Latch, G.C.M., Nott, H.M. and Tapper, B.A., 1993. Effect of water deficit on alkaloid concentrations in perennial ryegrass endophyte associations. *Proc. 2nd Int. Symp. Acremonium/Grass Interactions*, Hume, D.E., Latch, G.C.M. and Easton, H.S. (Eds), AgResearch, Palmerston North, New Zealand. Pp 67-71
- Fletcher, L.R., 1993. Heat stress in lambs grazing ryegrass with different endophytes. *Proc. 2nd Int. Symp. Acremonium/Grass Interactions: Plenary Papers*, Hume, D.E., Latch, G.C.M. and Easton, H.S. (Eds), AgResearch, Palmerston North, New Zealand. Pp. 114-118
- Prestidge, R.A. and Ball, O.J-P., 1993. The role of endophytes in alleviating plant biotic stress in New Zealand. *Proc. 2nd Int. Symp. Acremonium/Grass Interactions: Plenary Papers*, Hume, D.E., Latch, G.C.M. and Easton, H.S. (Eds), AgResearch, Palmerston North, New Zealand. Pp. 141-151
- Popay, A.J. and Rowan, D.D., 1993. Endophytic fungi as mediators of plant insect interactions. Pp. 83-103 *In: Insect-Plant Interactions*. Bernays, E.A. (Ed). Volume 5, CRC Press, Ann Arbor.
- Roylance, J.T., Hill, N.S. and Agee, C.S., 1994. Ergovaline and peramine production in endophyte-infected tall fescue: independent regulation and effects of plant and endophyte genotype. *J. Chem. Ecol.* 20: 2171-2183
- Woodburn, O.J., Walsh, J.R., Foot, J.Z. and Heazlewood, P.G., 1993. Seasonal ergovaline concentrations in perennial ryegrass cultivars of differing endophyte status. *Proc. 2nd Int. Symp. Acremonium/Grass Interactions: Plenary Papers*, Hume, D.E., Latch, G.C.M. and Easton, H.S. (Eds); AgResearch, Palmerston North, New Zealand. Pp. 100-102