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RYEGRASS STAGGERS: A ROLE FOR FUNGAL TREMORGENS

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SUMMARY

Results of an investigation into possible involvement of fungal tremorgens in the aetiology of ryegrass staggers (RGS) are presented. Effects produced by administration of penitrem A, verruculogen, and fumitremorgin B to sheep were similar to signs of RGS. *Penicillium* species capable of producing these tremorgens (penitrem A by *P. crustosum* and verruculogen and fumitremorgin B by *P. simplicissimum*) were isolated from pastures on which RGS occurred and also from dung taken from affected sheep. When cultured and dosed to sheep, effects similar to signs of RGS were again produced. Two tremorgenic species of *Aspergillus* have also been isolated from pasture. It is suggested that synergism between tremorgens may be a feature of the RGS syndrome. Although tremorgens were not detected in pasture samples, the results are consistent with a causal role for fungal tremorgens in RGS.

INTRODUCTION

Fungal tremorgens are a class of mycotoxins. Chemically they are indoles. When dosed to common laboratory animals and some farm animals, they cause sustained tremors, irritability, ataxia and tetanic convulsions (Wilson, 1971; Cysewski, 1973). These effects are similar to signs of ryegrass staggers (RGS) observed in sheep (Cunningham and Hartley, 1959; Keogh, 1973). Also, as in RGS (Munday and Mason, 1967), no gross structural lesions of the nervous system accompany the dramatic overt effects.

An association between RGS and fungi was first suspected more than 70 years ago (Gilruth, 1906), but successive investigations failed to reproduce the disorder when suspect fungal species were tested (Cunningham *et al.*, 1944; Thornton, 1964; Latch, 1972). In 1976, however, di Menna *et al.* produced in sheep and calves signs similar to RGS by dosing the animals with mycelium from cultures of a soil-inhabiting fungus, *Penicillium cytopium* Westling, and they postulated that the causative agent was a fungal tremorgen. A survey of the biological properties of the

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then known mycotoxins had led the present authors to conclude independently that fungal tremorgens might be the causative agents of RGS and a study of this hypothesis was commenced.

The investigation comprised two phases: an initial feasibility study in which authentic tremorgens were dosed to sheep and responses recorded; and attempts to obtain evidence of (a) the presence of tremorgen-producing fungi and/or tremorgens in environments in which RGS was occurring, and (b) the acquisition of such compounds by affected animals.

This paper summarizes results obtained.

EXPERIMENTAL OBSERVATIONS

EFFECTS OF TREMORGENS ON SHEEP

Authentic tremorgens penitrem A, verruculogen, fumitremorgin B and paxilline were used. On a dose rate basis verruculogen was the most and paxilline the least potent. Responses to mixtures of penitrem A and verruculogen (10:1 and 1:1 given intravenously) were more prolonged than when these tremorgens were given singly.

The behavioural responses to both intravenous and oral dosing included tremors, shaking, dyspnoea, ataxia and convulsions — responses which are commonly observed in sheep affected by RGS. This indicated that involvement of fungal tremorgens in the aetiology of RGS was feasible.

OCCURRENCE OF TREMORGEN-PRODUCING FUNGI AND TREMORGENS IN PASTURES

Tremorgen production had been recorded only for some *Penicillium* and *Aspergillus* species (Ciegler and Pitt, 1970; Yamazaki *et al.*, 1971): As aspergilli are rarely isolated from pasture samples (Latch *et al.*, 1976) attention was first directed towards the penicillia. Samples of herbage and soil from "toxic" pastures, as well as dung from affected animals, were screened for the presence of penicillia. Small samples of herbage were also screened for known tremorgens.

Of 23 *Penicillium* species isolated from herbage, soil or dung, two were shown to produce tremorgens (Gallagher *et al.*, 1977). *Penicillium crustosum* Thom* isolates S-17 and S-27 produced

**Penicillia* identifications were made by Dr J. I. Pitt, CSIRO, Division of Food Research, Sydney.

penitrem A when grown on 'Mapua' oats (*Avena sativa* L.), while *P. simplicissimum* Oudemans (*P. piscarium* Westling) isolates S-6 and S-31 produced verruculogen. Isolate S-6 also produced fumitremorgin B (Gallagher and Latch, 1977).

When cultures of the 23 *Penicillium* species were drenched to sheep, tremorgenic effects were produced in response to *P. crustosum* isolates S-17 and S-27, and to *P. simplicissimum* isolates S-6 and S-31, but not to any of the other *Penicillium* isolates.

In subsequent studies, two species of *Aspergillus* isolated from pasture have been demonstrated to produce known tremorgens when cultured.

Known fungal tremorgens were not detected in the herbage samples.

ACQUISITION OF TREMORGEN-PRODUCING PENICILLIA AND TREMORGENS BY GRAZING SHEEP

Each of the four tremorgen-producing strains of *Penicillium* was isolated from dung taken from the rectum of affected sheep in the field (Gallagher *et al.*, 1977). Thus, spores, at least, were being acquired by those animals.

In an attempt to obtain direct evidence of acquisition of tremorgens themselves, blood was taken from normal sheep and from sheep affected by RGS. The plasma was screened for the presence of known tremorgens; none were found in any samples.

To gain information regarding the possible behaviour of tremorgens in the blood, paxilline was administered intravenously to two sheep being fed hay indoors. The mycotoxin was found to disappear quickly from blood plasma, its half-life being 18 min. Signs of intoxication were still present when the tremorgen could no longer be detected.

Failure to find tremorgens in the plasma of sheep suffering from RGS could be due to one or more of the following: (a) A high removal rate similar to paxilline; (b) tremorgens are acquired only intermittently, as is likely to be the case in the field; (c) synergism between tremorgens is occurring and the mycotoxin levels may be too low to detect.

SYNERGISM

Synergism between tremorgens has been demonstrated in small animals (Wilson *et al.*, 1968; Ciegler, 1975). Three observations in this study suggested the occurrence of synergism:

- (1) The prolongation of response times when mixtures of penitrem A and verruculogen were used at low dose rates, mentioned above.
- (2) In 3 out of 5 experiments, sheep dosed with mixtures of tremorgen-producing *Penicillium* cultures were more severely affected than those dosed with the individual cultures. (Keogh and Latch, unpublished data).
- (3) Of sheep which were dosed with *P. simplicissimum* strains, those given cultures of S-6, which produced both verruculogen and fumitremorgin B, showed much more marked responses than did animals given cultures of S-31, which produced only verruculogen (Keogh and Latch, unpublished data).

The opportunity for synergism to occur in the field is indicated by the fact that strains of *P. simplicissimum* and *P. crustosum* were commonly found together in the dung of sheep affected with RGS.

DISCUSSION

Direct evidence for the presence of tremorgens in pastures and acquisition by the animal was not obtained. Although proof of acquisition was not demonstrated, clearly the opportunity for it to occur does exist.

The fungal tremorgen hypothesis offers plausible explanations for features of RGS observed in the field. Variation in the occurrence or severity of the disorder may reflect variation in the rate of acquisition and whether one or more tremorgens are being acquired. These in turn could be affected by factors such as interactions between the environment and toxin production, and the distribution of fungal species and grazing behaviour. Thus apparent correlation between grazing urine patches and the occurrence of RGS (Keogh, 1978) may be explained by two observations: first, that N added to cultures stimulates tremorgen production, and, second, that the *P. simplicissimum* isolate S-6, which produces both verruculogen and fumitremorgin B, grows vigorously on urine-scorched herbage (Keogh and Latch, unpublished).

The weight of the evidence collected so far is consistent with a causative role for fungal tremorgens in RGS.

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