Potential harmful effects of high endophyte content in perennial ryegrass on companion legumes

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Summary. Radicle elongation in *Trifolium* species was significantly reduced when they were grown in the presence of senescent, endophyte colonised, ryegrass vegetation, relative to endophyte-free vegetation. This was found in a pot trial together with laboratory bioassays, where the concentration of phytotoxins was found to be important in determining the response level. Phytotoxicity of the vegetation remained despite weathering of the vegetation in the field for several months. Retarded radicle elongation has significant implications for maintaining the annual legume density in pasture.

Introduction

Legumes are a vital component of pasture in the temperate regions of Australia because of the need for nitrogen fixation and to improve the feed quality of herbage. The majority of pasture in south eastern Australia contains either subterranean clover, *Trifolium subterraneum,* or white clover, *Trifolium repens,* and the most commonly sown grass is perennial ryegrass, *Lolium perenne.*

Results from a field experiment sown at Hamilton, Victoria, in autumn 1985 showed that the density of subterranean clover plants declined rapidly where there was a high frequency of ryegrass plants containing an endophyte, *Acremonium lolii,* but high clover density was maintained longer when the frequency of endophyte containing ryegrass plants was low (2). However, the direct causal mechanism responsible for these differences was not examined. In New Zealand, high frequency of this endophyte in perennial ryegrass resulted in an allelopathic effect on white clover which reduced stolon density (8). This result could not be ascribed to the impacts of defoliation or competition from the ryegrass.

To further examine potential allelopathic effects from endophyte in perennial ryegrass, the experiments described below were conducted using both a simplified bioassay system, to reduce the number of confounding factors which could affect the early growth of legume seedlings, and seed sown into soil to create a biological system that more closely resembled field conditions.

Methods

Experiment I

Dry mature culms were collected in mid-January 1990 from perennial ryegrass plants, cv. Ellett, grown in field plots. Seed had been shed from most of the culms prior to collection. The plots had been sown in 1989 with seed, 82% of which contained endophyte (E+) or with nil endophyte content (E-), however, in 1990 the plots were tested for the presence of endophyte and 96% of the plants were found to contain endophyte in the E+ plot, while 22% of plants in the E- plot contained endophyte (J.Z. Foot, pers. comm.). The herbage was air-dried and stored at room temperature. A crude aqueous extract was produced by chopping the herbage to 2 cm lengths and soaking it in distilled water for 10 h in a refrigerator (4?C). The extracts were then filtered through No. 1 qualitative filter paper before being applied to the bioassay assemblies, which were then stored in the dark at 20?C for five days. Distilled water was used as the control solution.

The seeds used in the bioassay were obtained from commercial seed lots of subterranean clover cv. Enfield and white clover cv. Irrigation, three accessions of each species were compared. Each bioassay assembly was prepared by wrapping a rectangular sheet of filter paper (17x15 cm, Ekwip grade R4, Robert Bryce and Co.) around a 2.5 cm diameter and 15 cm long, stoppered glass tube. During the wrapping 15 seeds were placed in a line on the filter paper. Thus, when finished, the seeds were arranged around the circumference of the tube 4 cm from its top and between two layers of filter paper. One rubber band was placed directly under the line of seeds, and another rubber band was placed 2 cm

from the bottom of the tube. The filter paper of each assembly was thoroughly soaked with extract. The seedlings were removed from the assemblies 5 days later when their radicle lengths were measured. Use of these assemblies ensured that the seedlings were in close proximity to potential phytotoxins throughout the germination period, and that the radicles of the seedling grew straight so that the lengths could subsequently be easily and accurately measured.

Pot trials were conducted on one accession of each species, chosen at random from the three available. Soil was collected that had not supported plant growth for 3 years and was thus assumed to contain low levels of phytotoxins. Pots (10x10 cm) were filled with 900 g of soil. Straw from the same sample used in the bioassay was chopped to 2 cm lengths, 16 seeds were placed on the soil surface and covered with 0.5 g of straw which was pressed lightly onto the soil surface. A further 4.5 g of straw was then placed on top. The pots were watered with 50 ml of distilled water to wash any phytotoxins from the straw into the soil. Further watering with 10 ml followed four times a day for the next five days then the seedlings were washed out of the soil and their radicle lengths measured. There were four replicates in both parts of the above experiment and a two-way analysis of variance was used to compare differences between the treatment means.

Experiment 2

Dry mature culms were collected in early January and early March 1991, from the same plots as in Experiment 1. This herbage was freeze dried and stored in a freezer in the presence of a desiccant to minimise the oxidation process which could reduce phytotoxins (9). A crude aqueous extract was produced after grinding the herbage through a mill fitted with a 2 mm screen, and then soaking it in distilled water for 10 h in a refrigerator (4?C). The extracts were then filtered as in Experiment 1. A dilution series was generated on a logarithmic scale from 0 (or undiluted extract) through 1:3.2, 1:10, 1:32, 1:100, 1:200, and 1:320, resulting in seven concentrations of extract which were applied to bioassay assemblies as in Experiment 1. Distilled water was used *as* the control solution. The seeds used in the bioassay were obtained from commercial seed lots of subterranean clover cv. Trikkala, and were graded to create a sample of uniform size. There were four replicates and an analysis of variance was used to compare the differences between the E+ and E- treatments.

Results and discussion

Experiment 1

Germination was not affected by the extracts, which is consistent with earlier results (6,1) where root growth of young seedlings was sensitive to phytotoxins but germination was relatively insensitive.

In the bioassay each seed accession responded similarly to the E+ and E- extracts, but the extent of each response varied. This was attributed to seed size which varied between the accessions, with large seeds consistently giving rise to longer radicles. There was no significant reduction in the radicle length of the subterranean clover. There was a significant reduction in the radicle length of the white clover ($P<0.05$) in the E- treatment relative to the E+ treatment (see Table 1), where a 25% reduction occurred. These results with white clover were unexpected and inconsistent with other results obtained in this laboratory (7) where E+ solutions inhibited radicle growth of white clover.

The pot experiment showed a significant decline of subterranean clover radicle length in the E+ treatment relative to the E- ($P<0.05$), a reduction of 11%. The white clover showed no significant reduction in radicle length.

Bioassay results are the mean of the three seed accessions

Experiment 2

The dilution series produced a curvilinear response similar to that observed by others (5) where high concentrations produced severe retardation of radicle elongation (Table 2). In the E+ treatment, all dilutions except treatments 1:100 and 1:32 had significantly reduced radicle elongation relative to the control (l.s.d (P=0.05) of 7.0 for comparison of treatments with the control). That the result of the 1:320 dilution E+ treatment was less than the control suggests that further dilution may have provided useful information. The E- treatment produced a curve that was not significantly different to the control value, except when undiluted leachate was used.

There was a significant reduction (P<0.05) in subterranean clover radicle length in the E+ treatment (mean 40 mm) relative to the E- treatment (mean 47 mm). The results from the two harvest dates were not significantly different to each other.

Table 1. Radicle length of clover seedlings (mm) at five days age.

Endophyte treatment	Bioassay[a]		Pot	
	Sub-clover	White clover	Sub-clover	White clover
High endophyte (E+)	41	16	31	11
Low endophyte (E-)	42	12	35	13
Water Control	44	17		
l.s.d. (P=0.05)	4	2	3	4

The absence of retardation of the radicles in the low concentrations of the E+ treatment suggests that novel Acremonium endophytes, those identified with an ability to produce low concentra- tions of specific bioactive compounds (3), could play a role in not only preventing retardation of radicle elongation but possibly stimulating elongation. The lengths of the hypocotyls of the seedlings were not measured as the phytotoxins present have nil or small effects on elongation of this component of the legume seedling (P. Quigley, unpublished data).

The phytotoxicity of chemicals leached from the ryegrass was maintained despite several months of weathering in the field, and may well be maintained through to the autumn break. The concentrations of several compounds produced by endophytes and by plants in response to presence of Acremonium endophyte may have considerable variability (3). This variability is likely to also occur in the specific biochemical/s responsible for the phytotoxicity seen here, and could strongly influence the degree of phytotoxicity of extracts derived from different grass/ endophyte combinations. The identification of chemical agents, as well as their mechanism of production requires investigation. This study confirms that the genotype of the legume can also modify the response to specific phytotoxins; the subterranean clover cultivar Enfield has been shown to be less sensitive than cultivar Trikkala to phytotoxins (F. Snell and P. Quigley, unpublished data). Variation caused by plant genotype is consistent with other studies (4).

Table 2. The effect of leachate dilution on radicle length of subterranean clover seedlings at five days age. Mean of both harvests.

Endophyte treatment	Dilution (mm)						
	Nil	1:3.2	1:10	1:32	1:100	1:200	1:320
High endophyte (E+)	30.7	40.8	41.7	42.3	44.1	40.4	38.3
Low endophyte (E-)	34.2	45.1	47.9	49.7	51.6	51.8	45.3
Control	49.0						

Retarded elongation of seedlings radicles has significant implications for maintenance of annual legume density in Australian pasture. At the beginning of the growing season, rainfall may often be erratic and the germinating clover seedling might have to withstand substantial soil water deficits as well as severe competition for moisture from grasses and weeds, particularly from perennial plants. We suggest that the phytotoxicity observed in these studies may contribute to the decline in legume plant density observed in the field experiments at Hamilton Victoria, and in New Zealand.

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