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Acremonium Fungal Endophytes of Tall Fescue and Perennial Ryegrass: Significance and Control

Among the most important and widely grown pasture grasses for cattle in the United States and for sheep and cattle in New Zealand are tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.), respectively. Tall fescue, a forage as well as a turf and conservation grass, is grown on 12–14 million hectares, primarily in the eastern half of the United States from Canada to the Gulf of Mexico. In New Zealand, perennial ryegrass covers 7 million hectares that support 60 million sheep and cattle. Both grasses offer many advantages to farmers because they are adaptable to a wide range of soil and climate conditions, are persistent, and have generally good nutritive forage value. Unfortunately, animals grazing these grasses often show symptoms of fescue (summer) toxicoses or ryegrass staggers. It has been established that grasses infested with fungal endophytes (*Acremonium* spp.) are responsible for the observed toxicosis symptoms (Table 1). Summer toxicosis in cattle and ryegrass staggers in sheep cause combined losses in animal productivity estimated in the hundreds of millions of U.S. dollars annually.

It is now possible to establish pastures with little or no endophytes in them, and animal production from these pastures is greatly enhanced (6). However, the unique mutualistic relationship between fungus and host may complicate the use of the "improved" endophyte-free cultivars. The presence of endophytes in grasses can confer resistance to various insects (4,10), enhance plant growth (9,11), and prevent overgrazing by herbivores (*unpublished*). These desirable stress tolerance characteristics of infested grasses should not be overlooked, as they may be required for continued persistence of the grasses.

In this paper we discuss various aspects of the biology, detection, incidence, dissemination, and control of the endophytes in tall fescue and perennial ryegrass as well as the significance of the mutualistic relationship between fungus and host grass.

Relationship of Endophytes to Maladies in Animals

In 1977, Bacon et al (1) reported the close association of endophyte-infested tall fescue and the incidence of fescue toxicity in cattle. The endophyte was identified as *Sphacelia typhina* (Pers.) Sacc., the imperfect state of *Epichloë typhina* (Pers. ex Fr.) Tul., but has recently been renamed *Acremonium coenophialum* Morgan-Jones & Gams. In Kentucky, studies have shown that cattle feeding on forage from endophyte-infested tall fescue pastures had lower average daily weight gains (ADG) and lower milk production than cattle feeding on endophyte-free tall fescue or tall fescue pastures infested with low levels of the endophyte (Table 2). Symptoms of summer toxicosis have been assumed

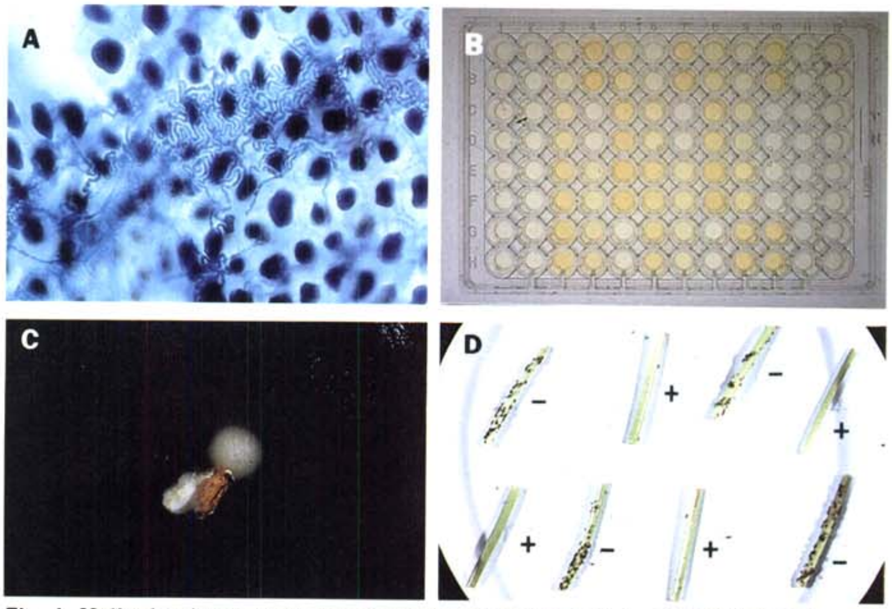

Fig. 1. Methods of detecting *Acremonium* endophytes: (A) *A. coenophialum* in seed stained with aniline blue; starch aleurone cells also stained blue (×75). (Courtesy E. Clark, Botany Department, Auburn University, Auburn, AL). (B) ELISA plate, with each sample replicated in adjoining wells; yellow samples are positive. (C) *A. lollae* isolate growing from seed on and in potato-dextrose agar incubated for 3 weeks. (D) Aphids (*Rhopalosiphum padi*) feeding preferentially on endophyte-free (−) rather than endophyte-infected (+) tall fescue stem sections; aphids (200) and stem sections were placed in petri plates and incubated for 18 hours.

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Table 1. Toxicosis symptoms of animals grazing *Acremonium*-infected pasture grasses in the United States and New Zealand

Country Grass	Animals	Symptoms: Known	Symptoms: Suspected
United States			
Tall fescue	Cattle	Reduced daily weight gains; elevated body temperature; elevated respiration; excessive salivation; rough hair coat; seek shade and water; reduced serum prolactin levels; reduced milk production	Agalactia; reduced conception
	Sheep	Reduced daily weight gains; reduced milk and prolactin levels	Reduced conception
	Horses		Agalactia; reduced conception
New Zealand			
Perennial ryegrass	Sheep Cattle Horses Deer	Reduced neuromuscular function, neck and limb tremors; reduced daily weight gains; reduced prolactin levels	Reduced conception

Table 2. Average daily weight gain of calves and milk production of lactating cows feeding on forage from *Acremonium*-infected and noninfected cultivars of tall fescue[t]

Animals	Forage: Infected (57%) Ky-31	Noninfected Ky-31	Infected (6.5%) GI-320	Noninfected Kenhy
Calves[u]				
Daily weight gain (kg)[v]	0.45[w]	0.70	0.89	0.86
Daily weight gain (kg)[x]	0.77 a[y]	1.07 b	...	...
Cows[z]				
Forage dry matter intake (kg/day)	7.2 a	8.7 ab	9.5 bc	10.4 c
Milk production (kg/day)	17.2 a	20.7 b	21.0 b	21.2 b

[t] Unpublished data, Animal Science Department, University of Kentucky, Lexington.
[u] Ten calves per treatment per pasture.
[v] Over 112 days (19 April–9 August 1983); average daily temperature, 26.7 C.
[w] Orthogonal contrasts were infected Ky-31 vs. noninfected Ky-31, Kenhy, and GI-320 ($P = <0.01$); noninfected Ky-31 vs. GI-320 plus Kenhy ($P = <0.01$).
[x] Over 42 days (11 November–22 December 1983); average daily temperature, 7.9 C.
[y] Mean values in a row followed by the same letter are not significantly different ($P = 0.05$).
[z] Five cows per treatment individually fed green chop forage and 8.2 kg per cow of concentrate daily.

primarily to develop under heat stress (>25 C). The data in Table 2, however, indicate that ADG of animals grazing infested Kentucky 31 (Ky-31) tall fescue in the fall, when temperatures were much lower than in the summer, were less than those of animals grazing endophyte-free pastures. Animal performance data from Alabama have also indicated that steer ADG over 4 years was 66% higher on tall fescue pastures with low levels of endophyte infestation than on heavily infested pastures. The chemicals that cause this fescue toxicity syndrome have not yet been identified.

A similar type of association between the endophyte in perennial ryegrass, identified as *A. loliae* Latch, Christensen & Samuels, and the incidence of ryegrass staggers was noted in 1981 by Fletcher and Harvey in New Zealand (3). Animals with this disorder appear normal until disturbed, whereupon they run with a stiff gait, stagger, fall, and have severe muscular spasms. They may roll down hillsides while having these spasms and drown in streams or marshes. Trials have shown that affected animals recover when they are transferred to noninfested perennial ryegrass pastures. The chemicals implicated in this disorder have been identified as tremorgenic neurotoxins, called lolitrems (5). The lolitrems are related to the known tremorgenic mycotoxins—aflatrems, penitrems, and janthitrems—in terms of structure, biogenesis, and biological effects.

Biology and Detection

A. coenophialum and *A. loliae* are true endophytes, completing their entire life cycles within the plant with no external sign of infection. Spores of the two *Acremonium* species have never been found on or in plants, although conidia may be produced in artificial culture. Both endophytes are seedborne, with their mycelium closely associated with the aleurone layer. During germination, the fungus invades the starchy endosperm and can be readily detected in the seedlings 3–5 weeks later. Most of the mycelium forms in leaf sheaths and seeds, with smaller amounts in stems and crowns, little in leaves, and probably none in roots. When plants are dormant, the fungi are confined to the apical meristem region; when plant growth resumes in the spring, the fungi grow intercellularly, without penetrating or apparently damaging the host cells. After growth of the flowering tiller starts, the mycelium grows in the culm pith to the flower panicles. Possibly, mycelium is also in the flower primordia before the inflorescence develops.

Attempts to artificially transmit the endophytes to mature plants by spraying macerated cultures of the fungi or by inoculation via wounds have not been successful. G. Latch and M. Christensen (*unpublished*) have fulfilled Koch's postulates by inoculating meristem tissue of 1-week-old seedlings of tall fescue and perennial ryegrass with their respective endophytes.

Viability of *Acremonium* mycelium in seed declines as the seed ages (8,13). Most endophyte-infected seed that has been stored in seed warehouses for 2 years contains little or no viable endophyte. This loss of fungus viability is retarded by low temperature and low humidity during storage. Endophyte-infected perennial ryegrass seed stored at 0–5 C and near zero humidity for 15 years has been found to contain living endophyte mycelium. Viability of the endophyte in infected tall fescue seed stored for 7–11 months at 21 C decreased to zero, whereas the viability in seed stored for 27 months at 10, 6, and −20 C was 30, 90, and 90%, respectively (13).

Endophytes can be detected in plants by staining the mycelium in leaf sheaths, stems, and seeds (1) (Fig. 1A) or by enzyme-linked immunosorbent assay (ELISA) (7) (Fig. 1B). The staining method clearly shows the coarse, mostly unbranched, convoluted intercellular nature of the mycelium (Fig. 2). In seed, however, dead fungus cannot be distinguished from living mycelium by either method. To detect viable endophyte, infected seed must be plated on agar (Fig. 1C) or the seed can be germinated and the presence of mycelium determined by ELISA or staining when the plants are 3–5 weeks old.

Pyrrolizidine alkaloids (*N*-formyl and *N*-acetyl loline alkaloids) are produced only in infected tall fescue plants, and their presence has been used as a chemical means of detecting the endophyte (2). Similarly, perennial ryegrass can be tested for the presence of the lolitrems to indicate whether plants are infected with *A. loliae*.

Because endophyte-infected host grasses are resistant to certain species of insects, bioassays can be designed to test for the presence of the endophytes (Fig. 1D). While this method of detection would probably not be used for routine analyses, it has been used to screen for toxic compound(s) responsible for insect resistance in tall fescue and perennial ryegrass.

Incidence, Dissemination, and Geographic Origin

It has been estimated that more than 90% of the tall fescue pastures in the United States and the perennial ryegrass pastures in New Zealand are infested with the *Acremonium* endophytes. Although these same grass/endophyte combinations have been found in other countries, there have been few reports of fescue toxicity or ryegrass staggers in animals. One exception is the regular occurrence of ryegrass staggers in sheep in southeastern Australia.

Important epidemiological questions are concerned with dissemination of the endophyte and rates of increase in percentage of infected plants in pastures from year to year. Transmission of the endophytes in tall fescue and ryegrass appears to be limited to seed produced on infected plants. Studies in the United States and New Zealand do not indicate dissemination by wind, rain, pollen, or mowing, but viable infected seed can be disseminated through the feces of grazing animals (12). Hay made from endophyte-infested pastures may contain infected seed, and when this hay is fed to animals on noninfested pastures, some of the seed will fall to the ground and produce infected plants. A limited amount of evidence suggests that the level of endophyte infestation in a tall fescue pasture will not change if the pasture is managed as a closed system. For example, when replicated plots of Kenhy tall fescue with five levels of infestation ranging from 5 to 75% were managed for seed production for 4 years, no significant change occurred in the level of infestation in any of the plots (12). In contrast, large increases in the percentage of perennial ryegrass plants infected with *A. loliae* have been noted in experimental plots in New Zealand and the United States.

Of primary importance in understanding why the endophytes are present in pastures at varying levels of infestation is knowledge of the incidence of endophyte infection in the original plants from which the grass variety was derived. Also, information is needed on the seed production and seed storage practices when the pastures were established. Both tall fescue and perennial ryegrass—and presumably the endophytes—originated in Europe. The presence of *A. coenophialum* in seven of 13 tall fescue genotypes (ecotypes) from Poland suggests a European origin for this fungus. Ky-31 was one of the first varieties of tall fescue grown in the United States and is now the most common. This ecotype was discovered in 1931 on the Suiter Farm (established prior to 1890) in Menifee County, Kentucky, and was released as a variety in 1943. From the time Ky-31 was discovered until the time it gained widespread acceptance by farmers, virtually all the seed planted was from the current year's harvest (12). Endophyte viability in tall fescue seed is known to be

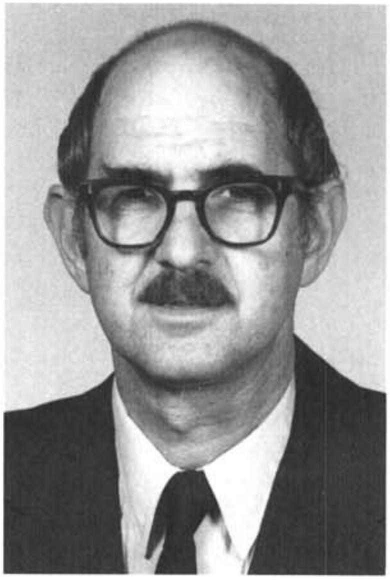

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Fig. 2. ***Acremonium coenophialum*** **in leaf sheath tissue of tall fescue. Mycelium stained with aniline blue (×300). (Courtesy E. Clark, Botany Department, Auburn University, Auburn, AL)**

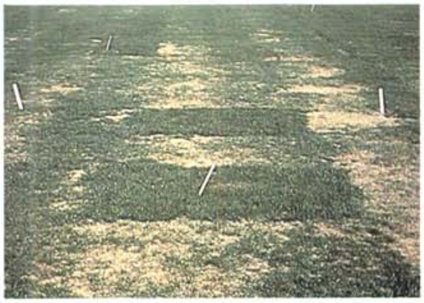

Fig. 3. Comparison of side-by-side (1 × 1.7 m) perennial ryegrass turf plots for resistance to sod webworm. Plants in plots infected with endophyte (green turf) are resistant to insect. (Courtesy R. Funk, Rutgers University, New Brunswick, NJ).

Fig. 4. Effects of ***Acremonium loliae*** **infection on growth of individual perennial ryegrass plants grown in the field. Plant on right is infected, plant on left is not infected. Original infected plant was divided and one portion was grown in sand containing 200 ppm of benomyl for 4 weeks. Tillers were transplanted to the field 18 months later and grown for 5 months.**

temperature-dependent and decreases or is lost during storage (13). Ky-31 seed was in such demand that most of the seed was never stored long enough for the endophyte to die, so the majority of the pastures are heavily infested. In contrast, a perennial ryegrass cultivar in New Zealand also was developed from plants infected with endophyte but was not popular with farmers, so seed was sometimes stored for several years before being sowed. Because endophyte viability declined during storage, pastures sown with this seed vary greatly in degree of endophyte infestation, some being endophyte-free.

Control Methods

The biology and epidemiology of the *Acremonium* endophytes suggest that any method that permanently reduces the percentage of infected seeds or the number of infected plants in established pastures would control the animal maladies associated with endophyte-infested grasses. Chemicals, especially the benzimidazoles and ergosterol biosynthesis inhibitors (EBI), effectively control the fungi in laboratory agar bioassays (13). Those fungicides have also been used as foliar applications or soil drenches to eradicate the endophytes in plants grown in pots in the greenhouse (8,13). However, no fungicide has been shown to permanently control the endophytes in the field. The level of infestation in tall fescue and the concomitant symptoms of summer toxicosis in cattle have been reduced temporarily (about 1 year) with the use of 3.3 kg a.i./ha or more of granular propiconazole and triadimefon (14). Because of the present high cost, the rates of EBI fungicides are not economical for commercial producers.

Endophyte in seed seems especially amenable to control by either chemical or heat treatment (8,13). Short-term heat treatment kills the endophytes in perennial ryegrass and tall fescue, but with a commensurate decrease in seed germination. Seed treatment of either host grass with certain EBI fungicides (triadimefon, triadimenol, dichlorobutrazol, and prochloraz) killed the endophyte in both greenhouse and field tests, but some phytotoxicity was noted.

The most desirable control is achieved by producing endophyte-free parent plants of grass cultivars (8). All future seed derived from these plants will also be endophyte-free. Seed lots of various tall fescue cultivars (e.g., Johnstone, Kenhy, Forager, Au-Triumph) produced from endophyte-free plants or plants with low levels of infection (<5%) have been released. Numerous states are using the staining method or ELISA to identify the presence of *A. coenophialum* and certify seed lots to have 5% or less infestation.

The means exist for establishing low endophyte-containing pastures of tall fescue and perennial ryegrass from certified seed lots. With tall fescue this appears to be a desirable goal, even though the labor and expense of destroying and replanting existing pastures seems high. Reestablishment would be warranted considering the potential increase in animal production, especially in the case of moderately to heavily infested (>30%) tall fescue pastures in the United States. In New Zealand, the case for establishing perennial ryegrass pastures with little or no endophyte is less clear. Perennial ryegrass pastures infested with *A. loliae* persist longer than endophyte-free pastures. Unless ryegrass staggers is a severe problem on their property, New Zealand farmers tend to accept endophyte-infected plants because they are more stress-tolerant than endophyte-free ones.

Host-Fungus Interaction and the Case for Mutualism

Mutualism has been defined as a close association between members of different species that benefits both. The fungus benefits by being protected within the plant and by dissemination through the seed. The plant's benefit is complex and involves increased tolerance of stress by endophyte-infested grasses. Stress tolerance is characterized as resistance by the plant to insect attack, overgrazing by herbivores, and by enhanced plant growth. All these tolerances have been found in *Acremonium*-infested grasses, although insect resistance and increased plant growth appear to be less pronounced in *A. coenophialum*-infested tall fescue than in perennial ryegrass.

Although insect resistance in tall fescue has not been noted in the field, laboratory and greenhouse studies show that feeding by the oat bird cherry aphid (*Rhopalosiphum padi* L.), greenbug (*Schizaphis graminum* Rondani), and large milkweed bug (*Oncopeltus fasciatus* Dallas) is reduced on endophyte-infected plants or extracts of infected plants. Infected tall fescue plants contain high levels (up to 0.6% or more by dry weight) of pyrrolizidine alkaloids. These alkaloids have been implicated as at least one of the toxic chemicals responsible for insect resistance (*unpublished*). In New Zealand, severe damage of endophyte-free ryegrasses by the Argentine stem weevil (*Listronotus bonariensis* Kuschel) has restricted the introduction of endophyte-free cultivars (10). The compound produced in endophyte-infected perennial ryegrass that discourages Argentine stem weevil attack has been isolated but not identified; it is not one of the lolitrems that cause ryegrass staggers. In the United States, Funk et al. (4) have demonstrated resistance to bluegrass billbug (*Sphenophorus parvulus* Gyllenhal) and to sod webworm (*Crambus* spp.) in turf-type perennial ryegrass containing high levels of endophyte (Fig. 3).

Another benefit the endophyte confers to some infected perennial ryegrasses is enhanced growth (Fig. 4) (9). While it has been suggested that growth is enhanced in infected tall fescue plants (11), the experiment previously described for Kenhy tall fescue showed no significant differences in plant growth and seed production. It is not clear what role different cultivars in geographic areas have on expression of enhanced plant growth.

The case for mutualism seems clear, but whether total separation of fungus from host grasses will affect survival of the grasses in different environments is not known. Severe damage by stress factors appears to be more likely in noninfected perennial ryegrass than in tall fescue. Lack of stress tolerance owing to absence of the endophytes may contribute to the decline or poor performance of these forage and turf grasses, although stress damage to the host grasses is probably not great enough to threaten species existence. The apparent lack of any form of dissemination other than by seed suggests that both *Acremonium* species require an infected host grass for continued survival. Thus, they may be regarded as ecologically obligate symbionts.

Progress and Problems

Since the discovery of the relationship between *Acremonium*-infected grasses and animal toxicoses during the last decade, considerable progress has been made by research workers in the United States and New Zealand toward understanding the fungal, plant, and animal components of this unique relationship. This has involved the cooperation of animal scientists, plant pathologists, plant physiologists, plant breeders, and entomologists—a truly multidisciplinary approach.

Unique opportunities exist for manipulation of stress tolerance properties in fungus-infected grasses. For example, the desirability of having *Acremonium* or other endophytic fungi in turf grasses to protect against insect attack, enhance plant growth, or induce disease resistance has been recognized by researchers. At least three new cultivars of turf ryegrasses, highly infested with *A. loliae* and resistant to some insects, will soon be released in the United States and New Zealand. Because turf grasses are seldom used as hay or pasture, the compounds toxic to animals that are produced by these infected turf grasses are of no importance. Both *Acremonium* endophytes have been successfully transferred to *F. rubra* L. This raises the possibility that insect resistance may be afforded this new host/endophyte combination and any other grass species that can be infected. If seed-transmitted in their new hosts, these endophytes may provide a means for developing turf grasses with better persistence than the endophyte-free grasses currently being grown.

Numerous problems of interest need clarification in order to better understand certain aspects of fescue toxicosis and ryegrass staggers as well as increased tolerance of stress by endophyte-infected grasses. These include knowledge of how the host and fungus interact to produce the chemical(s) responsible for insect and animal toxicoses; identification of these chemicals and elucidation of the nature of their toxic action in insects and animals; and better comprehension of various ecological and physiological aspects of the host/fungus mutualistic relationship.

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