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TIC VERT.

Endophytic Fungi in Perennial Ryegrass and Tall Fescue, and Their Association with Insect Resistance and Animal Performance

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Endophytic fungi are fungi that live within plant tissues, but not necessarily as parasites. Endophytic fungi have been known to occur within certain grasses for over one hundred years. However, the endophytes living within the tissue of tall fescue (Festuca arundinacea) and perennial ryegrass (Lolium perenne) (figure 1) received very little attention until recently, when two important discoveries were made. These discoveries are: (1) an association of endophyte presence with occasional periods of poor performance in animals grazing tall fescue and ryegrass pastures, and (2) the resistance of ryegrass containing the Lolium endophyte to several important insect pests. These discoveries have resulted in considerable interest in endophytes by both turfgrass and animal production specialists. Although there are still many questions to be answered about endophytic fungi, they may become an important tool in turfgrass pest management.

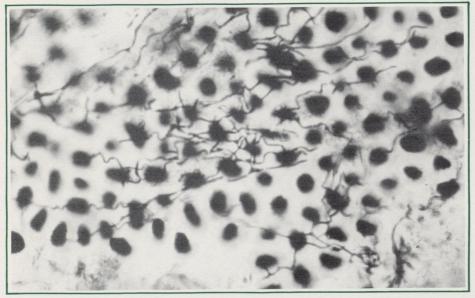


Figure 1.

Fungus threads (mycelium) grow in the intercellular spaces between cells of the aleurone cell layer (numerous oval cells) in the ryegrass or tall fescue seed. The endophyte grows between host plant cells in the seed, leaf sheath, and the pith of the flowering stems. The mycelium of the endophyte has not been observed to actually penetrate the host plant cells.

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Early Work

Sampson (1933) working at the Welsh Plant Breeding Station, noted that the Lolium endophyte in ryegrass was similar or identical to the fungus Epichloe typhina (Fr.) Tul, which causes the choke disease of fine fescue and some other grasses. However, when the endophyte is present in ryegrass, or tall fescue, it fails to produce choke disease symptoms, or any other readily visible symptoms. Sampson also observed that the endophyte was transmitted maternally by seed, as well as by vegetative propagation of an infected grass host. Neill (1940, 1941) and Lloyd (1959) conducted additional studies on the Lolium endophyte in New Zealand. They found that the endophyte was widely distributed throughout the country and was especially common in old pastures. They showed the endophyte lost viability, or died, in seed stored more than eighteen months. Very limited feeding trials showed no adverse effects of endophyte-infected plants on animal performance. No evidence was found that the endophyte had any effect upon its host plant either, leading to the conclusion that the fungus had little agronomic significance.

Animal Disorders Associated With Endophyte Presence

In 1977, work done by Bacon et al. in Georgia led to a reexamination of the endophyte in tall fescue. Studies were being conducted to identify the cause of "summer syndrome" or "fescue toxicity syndrome," a disorder that frequently occurs in cattle grazing tall fescue during hot summers. This

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Comments and Opinions

When Is Dioxin A Critical Problem?

We read a lot about dioxin, a chemical that has been present in many places for a long time and develops naturally as well as synthetically. Most articles are written by newswriters. One group that is seldom read or quoted is *Cast*. This is an association of scientists whose membership includes an impressive list of agronomists, horticulturalists, entomologists, animal scientists, veterinarians, plant pathologists, food technologists, weed scientists plus biologists and chemists associated with these fields. Information from this organization is seldom presented in the general press. The following is a summary statement from Dr. C.A. Black, Executive Vice President of Cast, who was an eminent soil scientist at Iowa State University until his retirement a few years ago.

The presence of dioxin in soil does not provide acceptable evidence of the existence of significant exposure of human residents in eastern Missouri adjacent to roads treated years ago with dioxincontaminated waste oil. Traces of dioxin are widespread in the environment. As a basis for determining whether the current exposure to dioxin is sufficient to be of concern, the first logical step would appear to be to determine the incidence of skin ailment known as chloracne in the human population. Other tests based upon easily conducted studies with animals would provide further verification of the significance of the hazard.

Whether you agree with the Cast statement, think of the recent government expenditure by EPA and the material loss of burying fifteen trainloads of turf fertilizer that possibly contained a teaspoon of dioxin as a contaminent in the silvex herbicide component. What will EPA propose for the road materials that people have been near or in contact with for years. Dioxin as a concentrated material must be treated with greatest respect, but when will our government agencies develop a better perspective when the concentration is one part per billion or one-tenth part per million. REE

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The pleasure of golfing should first feast the eye. An Old Bill Lyons Proverb.

Endophytic Fungi continued

summer syndrome results in poor weight gain, low milk production, an elevated body temperature, high rate of respiration, intolerance of heat, and overall poor performance. Because of the seasonal and sporadic nature of the syndrome a fungal toxin was suspected. Bacon et al. isolated the endophytic fungus, Epichole typhina (Fr.) Tul. (later renamed Acremonium coenophialum Morgan-Jones and Gams) from tall fescue pastures where cattle showing the summer syndrome were present. This study and several others have shown a definite association between endophyte-infected tall fescue and summer syndrome.

Recent studies in New Zealand by Fletcher and Harvey (1981), Mortimer et al. (1982) and others have shown that endophyte-infected ryegrasses may be responsible for another disorder, known as "Ryegrass Staggers" (RGS). This disorder affects sheep, cattle, and deer feeding on some overgrazed ryegrass pastures during periods of severe heat and drought stress. It involves temporary muscular tremors, causing the animal to stagger and fall, and occasionally causing injury and production losses. The animal will recover from RGS when a new source of feed is provided or when the pasture recovers from drought stress. These investigations found the endophyte to be most highly concentrated at the base of the plant in the leaf sheath, in the pith of flowering stems, and in seed. Heat and



drought stress appear to increase the amount of endophyte within an infected plant, as shown in Table 1. This helps explain why outbreaks of RGS occur, predominantly during periods of drought stress and heat, and during periods of over grazing, when animals are consuming the base of the plant. Unless these conditions are present, the widely distributed Lolium endophyte will not cause RGS.

Ryegrass is also widely grown as a highly desireable pasture grass in Northwest Europe. Although the Lolium endophyte is present in this area, no reports of RGS or similar disorders have been reported, probably due to the cool, moist climate. The authors are also unaware of any reports of ryegrass staggers affecting animals in the United States, where extensive acreages of annual ryegrass (Lolium multiflorum) are grown for winter grazing in the southeastern states. Nevertheless, using ryegrass varieties and seed lot with low endophyte levels would seem advisable. when the grass is to be used for forage purposes.

Toxins Associated With Endophyte Presence

While studies were conducted on the fungus responsible for these animal disorders, other scientists investigated the chemical nature of possible toxin(s) involved. In 1981 Gallagher et al. reported the isolation of a new group of neurotoxins, the lolitrems from ryegrass tissues and implicated them as the causative agents of RGS. When these neurotoxins extracted from ryegrass tissues were injected into mice, a tremor response similar to RGS symptoms, was obtained. It was later found that the toxins could only be extracted from ryegrass tissue that contained Lolium endophyte, further supporting the correlation betrween endophyte infection

Sanity is the conquest of truth without illusion.

and RGS (Gallagher et al., 1982). The neurotoxins could not be extracted from the fungus when it was grown on artificial media; this suggests that an association between an endophyte and its host plant triggers the production of lolitrems.

In tall fescue, endophyte presence is positively associated with the production of two pyrrolizidine alkaloids, N-acetyl Ioline and N-formyl Ioline (Bush et al., 1982). The accumulation of these alkaloids may be influenced by the environment, or an interaction between the fungus and host may occur during the growing season to produce varying amounts of these toxic compounds. It is also possible that the toxins are not produced by the fungus but by the plant in response to the fungus (Bacon and Hinton, 1983). The plant may be producing compounds toxic to fungi; such compounds are called phytoalexins (Whittaker and Feeny 1971).

Insect Resistance in Perennial Ryegrasses Containing the Lollum Endophyte

During the recovery of the grazing trial set up by Mortimer et al. (1982. 1983) containing plots of endophytefree grass plants and plots of endophyte-infected grass plants of the same cultivar, an unexpected difference in regrowth was observed. The trial area had been very closely grazed and subjected to drought conditions. The plants containing high endophyte levels produced uniformly good regrowth while the endophytefree plants exhibited uniformly poor regrowth. Investigations into why this remarkable difference occured showed endophyte-free plants to contain five times more adult Argentine stem weevils (Listronotus bonariensis) than were found on endophyte-infected plants. The endophyte-free grass plants had been severly damaged by the weevil, accounting for their lack of regrowth. This insect pest can cause decreases in pasture production of more than twenty percent, and is considered a major pest of New Zealand's droughty regions (Kain et al, 1982).

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Endophyte

Further evidence to support the resistance of endophyte-containing ryegrasses to Argentine stem weevil is given by Prestidge et al. (1982). Their study showed that over a period of insect infestation, pastures not treated with insecticides had a significantly greater number of plants that contained endophyte than insecticide-treated pastures. Natural selection of endophyte-infected plants was occuring. which explains high percentages of endophyte-containing plants in older pastures and turfs. Weevil numbers and damage levels will gradually decline with aging of the pasture because of the increase in percent of endophyte-infected plants.

While this work was progressing in New Zealand, an unusual response to sod webworm (Crambus spp.) feeding was noticed on perennial ryegrass turf trials conducted by Mazur et al (1981) and Funk et al (1983a) at the New Jersey Agricultural Experiment Station, Adelphia, New Jersey. Some cultivars and selections showed high levels of resistance to the sod webworm, whereas other entries showed substantial damage. Resistant entries showed a lack of larval feeding and an absence of larvae from the soil beneath them. Maternal transmission of insect resistance was dramatically evident, suggesting a hypothesis of endophyte-enhanced resistance to sod webworms. When remnant seed lots from these turf trials were microscopically examined for fungal presence, the ryegrass entries showing

resistance to sod webworm had a high percentage of seed possessing the Lolium endophyte, whereas the susceptible entries contained an insignificant amount of this fungus. The presence or absence of the endophyte was also confirmed by ELISA analysis.

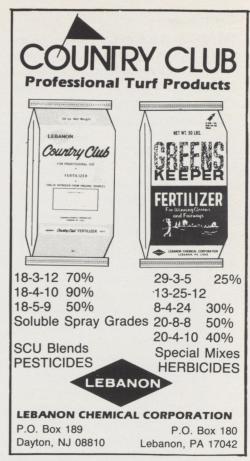
In other studies, seeds of forty-eight other cultivars and selections, including all entries entered in the 1982 National Ryegrass Turf Trials, were examined microscopically for endophyte infection. Of these forty-eight entries, twenty-one were free of endophyte whereas twenty-seven were infected. Rated on the basis of percent seed infected, five entries had ninety to one hundred percent, eight had fifty to eighty-nine percent, and fourteen showed less than thirty-three percent infected seed. Ryegrasses with high endophyte levels included Repell (GT-II), Citation II, HR-1, and Pennant.

Endophyte-enhanced resistance to the bluegrass bug (Sphenophorus parvulus Gyllenhal) in perennial ryegrass is also suspected (Funk et al., 1983a). In New Jersey turf trials, plots found to be resistant to this important insect pest had a lack of larval damage and a nearly complete absence of larvae. Maternal transmission of billbug resistance suggested a closer look and seed lots of both insect susceptible and resistant entries are now being examined for endophyte presence. The black beetle (Heteronychus arator) (A.V. Stewart, pers. comm., 1983) may also be controlled by the Lolium endophyte. Since endophyte-enhanced insect resistance is found in two dif-

 Table 1. Concentration of Lolium endophyte in plants sampled from a New Zealand ryegrass pasture.

	Relative Amount	Date		
Spring	6	Oct. 9		
	20	Nov. 2		
	44	Nov. 16		
	89	Nov. 30		
Summe	206	Dec. 30		
	67	Jan. 11		
	28	Feb. 8		
	18	Feb. 22		
Fal	26	Mar. 9		

Adapted from Fletcher (1983). Amounts of endophyte were determined using an ELISA test.



ferent orders of insects (Coleoptera and Lepidoptera), this suggests a broad-based mechanism of resistance. Disease organisims and nematode pests may also be affected. Endophytic fungi may well become a very useful tool for the plant breeder, not only for grasses but for other plants as well.

The Next Step . . . How Can This Endophyte Be Utilized?

With this newly acquired knowledge about endophytes in grasses many questions have arisin; the foremost being if and how they can be utilized. New Zealand scientists and farmers tend to feel that endophyte-infected grass is better than no grass for their animals to graze on, which might be the result if this important source of insect control is eliminated. Other ways of controlling RGS include improved grazing management practices and breeding livestock for resistance to endophyte. Sheep exhibit a large variation, shown to be highly inherited, in susceptibility to RGS.

U.S. farmers want the endophyte out of their tall fescue pastures. Because continued

Endophyte

tall fescue is the major pasture grass of the upper southeast U.S., and a statewide survey in Kentucky showed ninety-seven percent of the fields sampled to be infected with the endophyte (Lacefield et al., 1983), large numbers of livestock are involved. However, complete elimination of the endophyte may not be necessary as pastures with low percentage of endophyte-infected plants or pastures containing legumes in the mixture generally result in good animal performance. Measures are now being taken to produce endophyte-free seed so that pastures with lower endophyte levels can be established. Storing infected seed for approximately eighteen months at room temperature (cold storage prolongs fungus life many more years), or treating seed with systemic fungicides will result in endophyte-free seed (Latch and Christensen, 1982; Harvey et al. 1982). The treated seed can then be used as breeder lines and can be grown and multiplied for mass production of endophyte-free seed. Treating pastures with fungicides is not very effective and is cost prohibitive.

Since no cases of endophyteenhanced pest resistance in tall fescue have been found, controlling the endophyte should provide an effective, but not complete, solution to the summer syndrome problem. Moreover, it will be important to observe these endophyte-free tall fescue pastures for possible increases of damage from insect pests.

Turfgrass breeders see the endophyte as an important source of insect resistance which can be incorporated into new turfgrass varieties. Studies by Funk et al. (1983b) indicate that approximately ninety-five percent of the seed produced by an endophyteinfected plant will also contain the endophyte. Since the endophytecontaining plants are widely distributed in nature, and are also present in many commonly used varities, artificial inoculation of plants is not really crucial. However, this technique could prove useful if endophytic fungi with superior characteristics are isolated. Before the turfgrass breeder can proceed, more

information is sorely needed about how the fungus affects its host plant. From the earliest studies to the present, no evidence has been found of the endophyte having any adverse effects on yield or plant performance, but no comprehensive studies have ever really been done. With the use of endophytes in turfgrass varieties important modifications must be made in seed handling, storage, and labeling.

The endophytes of ryegrass, tall fescue, and many other grasses are not a new phenomenon. They are found in high frequencies in many pastures and turfs around the world. Their presence is not always associated with livestock problems as many environmental factors are also involved. Hopefully, with the proper use of these endophytes, turfgrass and even livestock managers can look forward to fewer pest problems and improved performance of their turfs, pastures, and cattle.

Acknowledgment

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Paul Des Champs, award chairman, presents the 1982 New Jersey Hall of fame plaque to Dr. C. Reed Funk. Recognized for his outstanding contributions to the field, Dr. Funk is Professor of Turfgrass Breeding at Rutgers University.

Dr. Funk's work has included release of Manhattan ryegrass; discovery of ryegrasses that resist sod webworms; and consistent improvements in tall fescue grasses. His awards include the Queen of the Netherlands 1982 Distinguished Guest List; the New Jersey Turfgrass Association's Achievement Award in 1976.

Abstract: Compaction and Irrigation Tests on Kentucky Bluegrass

by K.J. O'Neil and R.N. Carrow Agron J. 74:933-936. 1982

"Baron" was grown for two years on a Kansas soil with 19.6 percent sand, 60.7 percent silt and 19.7 percent clay. The sod was subjected to four treatments resulting from a factorial design with two levels of compaction (none and 30 passes per week with a roller) and two levels of irrigation. The set schedule was 3.8 centimeters (1.5 inches) water per week plus rainfall, and 3.8 centimeters when the tensiometer at 10 cm depth read—0.70 bar.

Soil compaction had no effect on the root weight or distribution. Visual quality, shoot density, verdure, and the percentage of total cover were reduced by compaction while total nonstructural carbohydrates (TNC) were unaffected. In the surface 3 centimeters of soil, compaction increased bulk density and moisture retention but reduced aeration porosity at -0.1 bar from 18.1 to 12.5 percent. Irrigation treatment had no effect on any of the soil's physical properties.

Without affecting turf quality, water use with a tensiometer was reduced by 28 and 48 percent on noncompacted and compacted areas, respectively, compared to set-schedule irrigated plots. Water use over a nine-day period in August indicated that the turf grown under the tensiometer-scheduled regime was physiologically or anatomically continued

Abstract continued from page 6

adapted to use less water even when it was available. The study showed this adaptation was not due to differences in vegetative or root growth.

Compaction reduced water use by 20 percent over the four-month study. During a nine-day period in August, compaction reduced water use by 3.5 to 11 percent for the tensiometer and set-scheduled treatments, respectively. This response appeared to be due primarily to altered moisture retention properties and reduced shoot growth. Thus, compacted and noncompacted sites should be irrigated on separate schedules.

Editorial Comments

The water saved with use of a tensiometer for irrigating Kansas turf as compared with a fixed quantity of water per week, shows the inefficiency of watering on a schedule. Using a green thumb or agronomic know-how might supplement the tensiometer or substitute for it in guiding water programs. Conjecture on the reduced water use on compacted plots is of interest. Were there differences in quantities of clippings which might have caused the difference? The author indicated the possibility of anatomical or physiological factors. Did the compacted soil develop a low oxygen level that reduced efficiency of water movement by the roots? If facilities had permitted it would be of great interest to continue a test of this type for a period of three to five years. This might show some good and bad long-term watering effects. REE

Endothal Goes Full Circle

A note on the use of endothall was published recently in some golf turf publications. The writers' conclusions were similar to mine more than twenty years ago. Compare the following (May 1983 Collaborator, New York State) with a statement from my 1960 publication.

May 1983 Collaborator

Controlling annual bluegrass (*Poa annua* L.) in bentgrass putting greens is a perennial problem. There are a number of preemergence herbicides that effectively control annual bluegrass. However, the continuing problem is to achieve control without damaging the bentgrass shoot or root system. Injury to the root system is particularly noticeable. Also, bentgrass damage can be aggravated by poorly drained soil and heat stress.

Australian golf course superintendents have tackled the problem differently. In 1976 several Australian superintendents began a long-term experimental program of endothall usage



that resulted in bentgrass putting greens free of annual bluegrass. Other superintendents using a similar control program are currently achieving the same results.

Endothall has been available for many years; however, the approach in the United States has always been to use it as a short-term treatment at high application rates with the objective of quick eradication. The Australians are doing just the opposite. They are pursuing a program of light, repeated applications appropriately scheduled. Continual application has resulted in complete control of annual bluegrass.

The endothall formulation being used is the sodium salt that contains 17.5 grams of active ingredient per liter. It is sprayed at a rate of 1/4 to 1/3 ounce to 2 1/2 gallons of water per 1,000 square feet. Typically, two applications at two to four week intervals are made in both spring and fall when temperatures are between 50° and 70°F. The result has been a gradual elimination of annual bluegrass over a two year period. There is evidence that perennial types of annual bluegrass are not as easily controlled with endothall as are the annual types.

It is important to avoid treatment when the turf is under heat, drought, or wear stress. Early morning or late evening treatments have been most effective. Endothall-treated bentgrass turfs possess substantially reduced wear tolerance; thus, application should be avoided five days before intense traffic is expected.

An error in application, such as applying when temperatures are too high or at too high a rate, can result in some foliar burn to the bentgrass. However, the turf readily recovers because endothall has no long-term soil residual that can cause toxicity problems for bentgrass, especially the root system.

The specifics on how such a longterm endothall program might be used in the United States are not established. The rates and timings may vary slightly depending on the location's specific conditions. Those interested in experimenting with such

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Endothall continued

a program should start on a nursery green with a rate of 1/8 ounce per 1,000 square feet and slowly increase the rate until the bentgrass phytotoxicity rate is established. Annual bentgrass control should be attempted only on greens that contain an adequate stand of bluegrass.

A concluding statement by Engel and Aldrich in WEEDS, 1960 Vol. 8:26-28

Two to three applications of 1/2 pound of endothall per acre applied at approximately two week intervals beginning with the start of early spring growth gave consistently good reduction of annual bluegrass (on a golf course fairway). This treatment also gave good clover control.

Obviously, we liked what we saw more than twenty years ago with endothall when the common name was spelled with one "I." Note that we suggested two to three applications of 1/2 Ib. per acre in early spring (before warm weather arrived). When endothall reached the market, it was often used at higher rates and after the start of warmer weather, which caused appreciable turf injury. These, along with the need for repeat applications, prevented the occasional users from accepting endothall in the USA. Within the past several years, I was informed that some experts in Oregon recommended endothall for annual bluegrass control in bentgrass at one pound per acre. For those who are looking for annual bluegrass control in bentgrass fairways, endothall is one of several things that might be considered. The cost of material is small and several applications give excellent clover control.

Stature comes not with height but with depth. Benjamin Lichtenberg Another statement from this earlier research is of interest and read as follows: "Maleic hydrazide reduced the number of annual bluegrass seedheads,but also seriously reduced the bentgrass content of the turf and allowed an increase of clover which forced discontinuance of this treatment (on fairways) after two years." **REE**

Kentucky Bluegrass has A Cure for Hot Weather Turf Depression

Hot, dry weather does more than cook the grass; it builds gloom and despair with each added spell of excessive heat. I am sure many of us are suffering from a sense of futility because we have had more hot days and drought than average to date.

Nature's cure for the Kentucky bluegrass lawn is the return of cool weather and some moisture which works like magic. Watching this recovery is one of my late summer-fall pleasures. Apply fertilizer a bit earlier and possibly use an extra application. Slice or scarify some seed into plate-size or larger voids. Even if the lawn is leaning toward the turf-type ryegrasses, inclusion of some good Kentucky bluegrass types keeps some of this grass on hand for good fall recovery and its other desired qualities. A balance of the two grasses is more desirable than either alone. REE

No Conclusive Evidence of 2,4-D Danger

A report by the American Medical Association's Advisory Panel on Toxic Substances concluded that, "There is still no conclusive evidence that the commercial herbicides 2,4-D and 2,4-5-T are mutagenic, carcinogenic or teratogenic in man, nor that they have caused reproductive difficulties in (humans)." According to the report, both herbicides break down rapidly in the soil" and are, therefore, of little environmental concern."

Pennsylvania Pesticide Report



