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THE USE OF MYCORRHIZAE IN THE ESTABLISHMENT AND MAINTENANCE OF GREENS TURF

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Most of the research on mycorrhizae, a beneficial association between fungi and plant roots, has focused on crop plants. Lack of research on turfgrasses may be due to an unsubstantiated theory that plants with fine roots and abundant root hairs do not benefit from mycorrhizae, especially if soil phosphorus is adequate. In fact preliminary investigations in this lab indicate that turfgrasses have up to 80% of their root systems colonized by mycorrhizal fungi.

SURVEY OF TURFGRASS SOILS FOR MYCORRHIZAL FUNGI.

Two different methods have been employed to determine what species of mycorrhizal fungi are present in soils from established plots of Agrostis palustris cv. Penncross, A. canina cv. Kingstown, and Poa annua.

1) DIRECT SOIL ISOLATION:
Turf soils are examined each month for spores of mycorrhizal fungi. This method indicates what species are present and their relative abundance in the soil at the time of collection.

2) POT CULTURE TECHNIQUE:
Because mycorrhizal fungi are obligate symbionts they can only be cultured when grown with a host plant ("pot culture"). Pot cultures of turf soils are established each month. These cultures are harvested after 4-6 months to check for spore production. Species of fungi vary in their ability to sporulate, and some species sporulate more readily in pot culture than in the field. Thus by combining data from pot culturing and direct soil isolation we will obtain a more complete survey of the fungal community in turf soils.

RESULTS:

Using the above methods, 24 species of mycorrhizal fungi have been identified (including 12 new, undescribed species) as being associated with greens turf. Four genera of fungi have been recovered including Glomus, Scutellispora, Acaulospora, and Entrophospora. In addition single species are being pot cultured. We plan to test the efficacy of different species on greens turf. Different seasonal trends in spore abundance and species composition were identified for each species of turfgrass.

MYCORRHIZAL INOCULATION EXPERIMENTS:

1) GREENHOUSE:

The mycorrhizal fungus Glomus intraradix produced a significant increase in shoot dry weight in both species of bent grass as compared to uninoculated plants. A mixed culture of mycorrhizal fungi isolated from turf soils did not increase shoot dry weight. Techniques to generate inoculum on a large scale are being investigated.

2) FIELD:

A small sand green (USGA specifications) was installed in the field in September, 1990. The effect of mycorrhizal inoculation with Glomus intraradix and different levels of phosphorus on A. palustris and A. canina will be evaluated. The green was divided into 288 square-foot plots using a plywood grid. Some plots are being used to examine the interaction of two different peats, sedge and sphagnum, on bent grass growth and mycorrhizal inoculation. A parallel study will be established in the greenhouse by December, 1990.

LABORATORY:

A new method of inoculating bent grass plants with mycorrhizal fungi under monoxenic conditions in liquid culture has been developed. Attempts to establish root organ cultures of bent grass have not been successful to date.

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ENDOPHYTES OF TURFGRASSES: NEW TOOLS AND APPROACHES

Executive Summary

This project was proposed and initiated by Dr. Peter Day, Center for Agricultural Molecular Biology (AgBIOTECH) and Dr. Reed Funk, Department of Crop Science, Rutgers University. Dr. Jane Breen, the post-doctoral researcher began work on the project on 1st. April 1990, in the laboratory of Dr. Michael Wilson (Professor, AgBIOTECH). Our goals are: (a) to produce a germplasm collection of fungal endophyte-infected grasses concentrating on *Poa* and *Agrostis* species; (b) to produce a collection of unifungal endophyte cultures for both classical and molecular analysis; (c) to produce endophyte-specific DNA probes for sensitive detection of particular species and/or isolates of endophyte by nucleic acid hybridization techniques and by recently developed polymerase chain reaction (PCR) amplification methods; (d) to use these and other molecular probes to characterize endophyte variability and produce RFLP maps as taxonomic aids in this field; (e) to develop gene transfer methods for fungal endophytes, as well as fungal transfer methods between different turfgrasses, to test for compatibility/incompatibility; and (f) to identify those genes responsible for insect repellant alkaloid biosynthesis and metabolism, particularly in beneficial endophyte-grass combinations.

Including material collected recently using USGA-funds and acquisitions from Dr. Funk's turf breeding program, we now have a germplasm collection of multiple selections of fourteen species of endophyte-infected turfgrasses, including some bentgrasses and bluegrasses (*Agrostis hiemalis*, *A. scabra, A. alba* and *Poa palustris*, *P. autumnalis, P. ampla*), in addition to a number of tall fescues, perennial ryegrasses and fine fescues. Our endophyte collection includes multiple isolates of eight species of *Acremonium* fungal endophytes which infect a broad range of grasses. A DNA library has been developed from *Acremonium starrii*, which will be used to provide probes for DNA 'fingerprinting' to evaluate and quantify genetic differences among endophytes, particularly in terms of those which confer insect resistance or have the ability to produce choke.

I. Turfgrass Germplasm Collection:

Our germplasm collections of endophyte-free and endophyte-infected turfgrasses are being used for molecular and classical experiments by groups in AgBiotech, Plant Pathology and Crop Science, as well as collaborators at other Institutions. All infected grasses are maintained in a greenhouse and are being introduced into the breeding program for field evaluation.

Our collection of endophyte-infected grasses includes multiple selections of fourteen species of grasses including *A. typhinum*-infected *Agrostis hiemalis*, and *A. typhinum*- or *A. starrii*-infected *Poa palustris* (Table 1).

II. Endophyte culture collection:

One of our first priorities was to develop a collection of endophytes from a wide range of grasses with different, agronomically important traits. To date we have multiple isolates of eight species of endophytes (Table 2). In most cases these are also maintained in their original hosts. The cultures are being used to evaluate endophyte variation using genetic fingerprinting as well as more traditional measurements such as differences in growth rate and sugar utilization.

In a parallel project (**Section VI**), these endophytes are also being screened for the presence of double-stranded RNA and plasmids.

Dr. David Huff (Crop Science Department, Rutgers) has been successful in using a number of these isolates to test for antibiosis to dollar spot.

Dr. James White is continuing to collaborate with us in evaluating the physiological and morphological differences between endophytes.

III. Screening for *Agrostis* and *Poa* endophytes:

The first approach to finding an endophyte in *Agrostis* and *Poa* spp. was to screen local field-collected grasses. Over seven hundred selections of bentgrasses and bluegrasses collected from Massachusetts to Kentucky have been screened for endophytes, in addition to several hundred *Poa pratensis* acquisitions which had been screened previously in the turf-breeding project.

In May we obtained a grant ($13,500) from the Rutgers Turf Research Fund for Dr. James White, a mycologist from Auburn University, Alabama, specializing in fungal endophytes, to work with us in developing and extending these collections. Dr. White suggested that field collections would be more efficient if based on examination of herbarium specimens. He examined all *Agrostis* and *Poa* in the Rutgers Chrysler herbarium. The selections were from North America, primarily New Jersey and New York. A number of species of *Poa* and *Agrostis* were found to be infected. Based on this survey he was able to go to the field and collect several selections of infected *P. palustris*, *P. autumnalis* (fowl bluegrass), *A. hiemalis*, *A. scabra* and *A. alba*. However, all Kentucky bluegrass (*P. pratensis*) and creeping (*A. palustris*), velvet (*A. canina*) and colonial (*A. tenuis*) bentgrasses were found to be uninfected. One herbarium specimen of redtop (*Agrostis alba*) from New Jersey was infected, but an uncooperative landowner meant that we have not yet been able to collect this in the field; two other sources of endophyte-infected *A. alba* were found by Dr. White.

Another approach was to examine collections of bentgrasses and bluegrasses from Europe or Asia closer to their center of origin. This was done by screening USDA plant introductions. Three selections of *Poa palustris* were found to be infected: two from Canada with *A. typhinum*, and one from Yugoslavia with *A. starrii*. None of the USDA *Agrostis* plant introductions examined to date (125 selections) were found to be infected.

Recently Dr. Breen screened a collection of over three hundred creeping bentgrass selections from Italy, France and the U.S. for endophytes (courtesy of Dr. Milt Engelke, Dallas). None were infected with fungal endophytes, however

bioassays for field resistance to fall armyworm indicated that many of the selections had natural, genetic resistance. Samples of 100 of these *Agrostis* spp. have been transferred to Rutgers for further analysis and assessment.

IV. Restriction Fragment Length Polymorphisms (RFLPs):

RFLPs (DNA fingerprints) are being used to characterize relationships between and within endophyte species and isolates, and between species with different agronomically important characteristics e.g. different degrees of choke production or insect resistance. To produce probes, DNA is extracted from an endophyte culture and cut with restriction enzymes. The resulting fragments are inserted into a suitable plasmid vector for amplification in bacteria. The endophyte DNA inserts are then used as probes for Southern blot hybridization of a range of endophytes. Alternatively, DNA probes based on repetitive, highly conserved, ribosomal RNA genes, spacers, or those used in forensic science (CAC-rich) can be used.

We have made limited progress in creating a library of genomic DNA from *A. starrii*, the endophyte in 'SR3000' hard fescue, in the plasmid vector pGEM-3Z. The DNA fragments inserted in these plasmids are being used as probes to detect homologies between and within different endophyte species, with the ultimate goal of developing an RFLP map and cluster analysis to evaluate degrees of relatedness among *Acremonium* species.

We have also begun sequencing some fragments of *A. starrii* DNA, since this will enable us to make and use oligonucleotides as primers for polymerase chain reaction (PCR) amplification. PCR is theoretically capable of detecting a single molecule of homologous endophyte DNA in a crude plant extract. This technology is in everyday use for other projects in Wilson's laboratory.

V. Related Projects:

Support from the USGA enabled us to obtain funding for two parallel, complementary projects, as well as to justify extra support for a summer sabbatical visit by Dr. J. White (**Section III**).

(1) "Screening for extrachromosomal genetic elements in *Acremonium* endophytes". This proposal to the Rutgers Turf Research Fund was approved at $122,000 over 3 years. In this project, endophytes collected and isolated by the USGA project will be analyzed for double-stranded RNA and for plasmids. Double-stranded RNAs have been associated with both hypervirulence and hypovirulence in yeasts (ds killer RNA) and in chestnut blight fungus (*Cryphonectria parasitica*), so these molecules could be associated with the ability to produce choke. A Ph.D. student holding a masters degree was recruited in September 1990 and has already been successful in isolating two double-stranded RNA molecules (2.0 and 2.5 kbp) from the endophyte *Atkinsonella hypoxylon,* associated with high choke production.

(2) In a second project, funded at $18,272 for two years by the National Turfgrass Evaluation Program, the 1989 fine fescues are being screened for *Acremonium* endophytes. The isolated endophytes will be added to the Rutgers

collection for inclusion in future tests and molecular analyses. The infected fine fescues will be evaluated for insect resistance at VPI & SU by Dr. D.G. Pfeiffer, an entomologist. Dr. Pfeiffer will also examine the compatibility of endophyte enhanced resistance with biological control by evaluating effects on a grass feeding mite and the important predatory mite *Amblyceus fallacis.*

VI. Future directions:

Since *Epichloe typhina* was reported in *Agrostis palustris* and *A. tenuis* in Great Britain (Bradshaw, 1959) we will try to obtain further information and material from plant breeders in Great Britain (Dr. Peter Hayes, Bingley, to be contacted). We have also tested techniques for inoculating an endophyte (*A. typhinum*) from blue fescue into endophyte-free clones of the same grass using conidial suspensions. In the event that we are unable to obtain a naturally occuring endophyte in *Poa pratensis* or in *Agrostis palustris*, we will attempt to inoculate artificially with endophytes from congeneric species or forms that seem to be widely adapted to a range of grasses.

Bradshaw, A.D., 1959. Population differentiation in *Agrostis tenuis* Sibth. II. The incidence and significance of infection by *Epichloe typhina*. New Phytol. **58,** 310-315.

Table 1.
Collections of Endophyte-Infected Turfgrasses

Grass species	Endophyte
Each genotype +/- endophyte; 4 pairs per grass species	
Perennial Ryegrass	*Acremonium lolii*
Tall fescue	*A. coenophialum*
Chewings fescue	*A. starrii*
Hard fescue	*A. starrii*
Blue fescue	*A. typhinum*
Individual field collected selections	
Poa palustris	*A. typhinum, A. starrii*
Poa autumnalis	*A. typhinum*
Poa ampla	*A. typhinum*
Agrostis hiemalis	*A. typhinum*
Agrostis scabra	*A. typhinum*
Agrostis alba	*A. typhinum*
Danthonia spicata	*Atkinsonella hypoxylon*
N.J. fine fescues	*A. typhinum*
Breeder/PI selections	
Poa palustris	*A. typhinum, A. starrii*
Lolium rigidum	unconfirmed
Lolium multiflorum	unconfirmed
Icelandic fine fescues	*A. starrii*

Table 2.
Collection of Endophytes in Culture

Endophyte	Host plant(s)
A. lolii	*Lolium perenne*
A. coenophialum	*Festuca arundinacea*
A. typhinum	*Poa* spp. *Agrostis* spp. *Festuca* spp.
A. starrii	*Festuca* spp.
A. chilense	*Daytylis glomerata*
A. chisosum	*Stipa lobata*
A. huerfanum	*Festuca arizonica*
Atkinsonella hypoxylon	*Danthonia spicata*

Table 3.
Rutgers Chrysler Herbarium Screening of *Poa* and *Agrostis* spp. for Endophytes

Species	#Infected/total	% Infected
A. palustris	**0/10**	**0**
A. tenuis	0/13	0
A. canina	0/4	0
A. exarata	0/4	0
A. hiemalis	12/31	39
A. perennans	9/16	56
A. stolonifera	0/14	0
A. borealis	0/11	0
A. arachnoides	0/1	0
A. alba	1/6	17
P. pratensis	**0/8**	**0**
P. longiligula	0/1	0
P. alsodes	9/14	64
P. languida	5/6	83
P. sylvestris	5/7	71
P. trivialis	2/11	18
P. canbyi	0/1	0
P. charxii	0/1	0
P. alpigena	0/1	0
P. saltuensis	0/1	0

Bold = those that were particularly sought.

Table 4.
Workshops and Meetings Attended

1. Workshop: "Hybridization Analysis". J. Breen. April 1990. Duke University, Durham, North Carolina.

2. Presentations:

Breen J. October 1990. The potential effects of *Acremonium* endophytes in orchard groundcovers on predators and parasitoids. Eastern branch E.S.A. meeting, Baltimore MD.

Breen J. October 1990. Variation in enhanced resistance to insects in *Acremonium* endophyte-infected turfgrasses. Texas A&M Exp. Sta. Dallas, TX.

Funk, R., White, R., and Breen J. November 1990. The use of *Acremonium* endophytes in turfgrass breeding. International *Acremonium* Endophyte Symposium, New Orleans, LS.

Breen, J. November 1990. Temperature and seasonal effects on the expression of *Acremonium lolii* enhanced resistance to greenbug, *Schizaphis graminum* (Rondani). International *Acremonium* Endophyte Symposium, New Orleans, LS.

Breen J. December 1990. Endophyte enhanced resistance to insects: Variation among insects, host plants, and endophytes. E.S.A. National meeting, New Orleans, LS.

Breen J. December 1990. Alternative methods of pest control: endophytes. Turf Expo. Atlantic City, NJ.

Table 5.
Personnel and Project Collaborators

P. R. Day, Ph.D.	Director, AgBiotech Center
R. Funk, Ph.D.	Professor, Crop Science
T.M.A. Wilson, Ph.D.	Professor, AgBiotech Center
J. Breen, Ph.D.	Post-doctoral Researcher, AgBiotech Center
B. Hillman, Ph.D.	Assist. Prof., Virologist, Plant Pathology
Chan-Seok Oh, M.S.	Graduate Student, Plant Pathology
J. White, Ph.D.	Assist. Prof., Mycologist, Auburn Univ., AL
D. Huff, Ph.D.	Post-doctoral Researcher, Crop Science
M. Engelke Ph.D.	Professor, TAES, Dallas, TX
D. Pfeiffer Ph.D.	Professor, Entomologist, VPI & SU, VA
S. Sun	Graduate Student, Crop Science